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An Experimental Study of the Life Cycles and Taxonomy of Allomyces[1]

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[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, no. 186.

[2] Part of this study was made at The Botany School, Cambridge University, England, during the tenure of a Fellowship granted by the National Research Council of the Rockefeller Foundation.

Introduction

Allomyces is a genus of filamentous, aquatic Phycomycetes of unusual interest and importance from the mycological as well as the general biological point of view. Certain species reproduce sexually by anisogamous planogametes, a unique condition among the water moulds, and exhibit a striking alternation of equal asexual and sexual generations. Studies of other species have revealed that there are moreover, within this single genus, three markedly different types of life cycle, one of which involves a regular process of encystment, an exact counterpart of which is not known at present in any related forms. Because of these and other characters of particular interest and because of the readiness and rapidity with which all species will grow in pure culture on agar media, *Allomyces* offers outstanding possibilities for experimental studies of both asexual and sexual processes of reproduction.

In a brief survey, the work of earlier investigators can logically be grouped as follows:

1. Early work, concerned only with the asexual stage, before the discovery of sexuality.
2. More recent work, concerned with discovery and study of the sexual stage.

EARLY WORK

The genus was established by Butler (1911) who discovered a single species, *Allomyces arbusculus*,* in India. Butler carefully described and figured the general morphology of this fungus, and although subsequent work has led to a more complete and detailed understanding, our present conception of the asexual stage of *Allomyces* agrees, in a large measure, with that first description. Less than a year later Barrett (1912) isolated and studied a closely similar fungus; he grew it in pure culture and, in addition to confirming most of Butler's morphological work, went into the cytology in some detail, describing for the first time the unique false septations in the hyphae, the character of the resistant sporangium wall, and the relation between the true nucleus and the nuclear cap. He was also first to observe germination of resistant sporangia in *Allomyces*, and for that matter in the Blastocladiales, and described the manner in which zoospores were liberated from these heavy-walled structures. His investigation was a most important addition to Butlers' earlier work.

No significant advances in our understanding of the genus were published between 1912 and 1929. Weston (1914) obtained a third isolate from soil collected in Alabama, U. S. A., and his studies of this form were in essential agreement with those of Butler and Barrett. Coker and Grant (1922)

* According to Article 72 of the International Rules of Botanical Nomenclature, generic names ending with *-myces* are masculine. Hence the specific name *arbuscula* Butler (1911) should be *arbusculus* to agree in gender with the generic name *Allomyces*.

investigated another strain which they found in North Carolina, while Coker and Braxton (1926) described a new species, *A. moniliformis*, also isolated from soil. Separation of this species from *A. arbusculus* was based primarily upon the length and arrangement of sporangia, but germination of resistant bodies was not observed and no further distinctions were brought out. Many other strains of *Allomyces* were collected, and these early isolations and studies, in which true sexual reproduction was not observed, are summarized under *A. arbusculus* (starred entries) and *A. moniliformis* in the list of isolates, pp. 81–84. The outcome of the researches briefly outlined above was a fairly thorough understanding of the morphology and asexual reproduction of *Allomyces*.

DISCOVERY OF SEXUALITY

Subsequent investigations were concerned more particularly with discovery of the gametophyte generation and description of sexual reproduction. The first indications of sexuality were discovered by Weston (1918) in a strain of *Allomyces* which he collected from a drainage gutter at Los Baños, Philippine Islands, in June 1918. When he revived this culture from dried material in the following year he made drawings of the plants which developed and noted that two types of "sporangia" were being formed: (1) large, ovoid to spherical, gray "sporangia" which liberated large, hyaline, less active swarmers, and (2) small, cylindric, orange "sporangia" which liberated small, active, pale orange swarmers. Fusions between the two types of swarmers were not seen but accompanying some of the drawings was the written query, "Are these gametangia and gametes?"

Unfortunately, this question was not answered until ten years later when Kniep (1929) published his concise account of sexuality in *Allomyces javanicus*. This paper is of fundamental importance, not only because it is the first clear-cut description of sexuality in the Blastocladiales, but more particularly because it portrayed a type of sexuality hitherto unknown in the fungi, i.e., copulation of anisogamous planogametes. In connection with his investigations of water and earth samples in Java, Kniep (1929) isolated a strain of *Allomyces* which, in vegetative structure and asexual reproduction, was very similar to *A. arbusculus* Butler. He discovered however, that certain plants bore male and female gametangia instead of zoosporangia on the hyphal tips and on this basis described his isolate as a new species, *A. javanicus*. Each chain or pair of gametangia was usually terminated by a male, with a female in the subterminal position. That is to say, the male gametangia were epigynous. Females and males differed markedly: the former, like zoosporangia, were unpigmented; the latter were somewhat smaller and bright orange. Male and female gametes were both motile, posteriorly monoflagellate, and morphologically similar to zoospores, but the males were distinctly smaller and more active than females and were colored

orange. Gametes fused to form biflagellate zygotes which germinated directly, without the period of rest so characteristic of almost all other phycomycetous zygotes.

That there was a regular alternation of equal gametophyte and sporophyte generations in *A. javanicus* was also demonstrated by Kniep (1930). He showed that sexual plants arose from zoospores discharged from resistant sporangia, while asexual plants arose from zygotes as well as from zoospores formed in thin-walled sporangia.

Another species of *Allomyces*, which Kniep (1930) collected in Bali but did not name, proved to be very similar in life history and general morphology to *A. javanicus* but differed markedly from the latter in arrangement of sexual organs. Pairs or chains of gametangia terminated with a female; males were formed subterminally and hence were hypogynous rather than epigynous as in *A. javanicus*. Weston's Philippine Island strain when reexamined (in 1929–32 by Weston, Sparrow and others, and in 1933 by the writer) was also found to have hypogynous male gametangia and to agree with Kniep's brief description of the isolate from Bali. A third form (isolate North Carolina 2 in the list below) collected near Raleigh, North Carolina, was studied by Hatch (1933), who discovered that here too male gametangia were hypogynous. His account of sexual reproduction fully confirmed earlier observations.

NEED OF A GENERAL SURVEY OF THE GENUS

At the time the writer undertook his studies in 1933, the morphological alternation of generations in *A. arbusculus* and *A. javanicus* had been clearly described, the structure and function of the reproductive organs borne on sexual and asexual plants were known, and the morphology and usual behavior of the four types of swarmers liberated by these organs had been carefully observed. But, though separate isolates had been investigated in detail over a period of twenty-two years (1911–33), no general survey or comparative study of a complete collection, including all the known species and a majority of the described strains, had ever been made. The discrepancies in description and nomenclature alone which had arisen during this time indicated the need of such a study. Moreover, resistant sporangia of *A. moniliformis* had not been germinated, and it was not known whether this and other forms of *Allomyces* all had life histories such as that of *A. javanicus* and *A. arbusculus*. For studies of life cycles therefore, and purposes of experimentation, the writer gathered together 51 isolates of *Allomyces* and worked out the developmental history of each. A comparative, experimental study of these forms extending over a period of six years has made it possible to determine which of their characters are most fundamental and least variable and can serve to distinguish species and group

them in clear-cut subgenera. So it is the purpose of the present paper to outline and explain the criteria which the writer has used in drawing up a scheme of classification for *Allomyces*. This study was a prerequisite for genetical and other experimental investigations being done by the writer and will, it is hoped, be of permanent value in future work which will surely be carried on with this unique fungus.

Materials and Methods

ISOLATES OF ALLOMYCES

All the isolates which have been reported since *Allomyces* was first discovered in 1911 are listed below. A complete list of this sort has not hitherto been published; it is included here because it will help to summarize the past studies of the genus outlined in the introduction, and it will also be very useful in the present paper for a number of other reasons. First, the exact localities given in the list can be correlated with the points, necessarily less precise, on the world maps (p. 139) showing geographical distribution of the various species. Second, the brief notes indicating the kind of sites where collections have been made should be useful in guiding future collectors. And third, the origin of each isolate is shown and references to reports or descriptions of each are listed here because it is most important in the present study, as well as for the coordination of future comparative work, to understand clearly which isolates in the writer's collection have already been examined and described by other investigators and which of these are type isolates. The 51 strains in the writer's own collection are distinguished in the list by bold face type; 33 were isolated from soils by the writer himself while 18 were obtained through the courtesy of other workers. This collection includes representatives of all the known species as well as many of the actual strains studied in detail by previous investigators.

Acknowledgments: The writer is very much indebted to all the many people through whose kind efforts it has been possible for him to obtain samples of soil from distant parts of the world. He is also particularly grateful to Professor J. N. Couch, Dr. B. B. Kanouse, Dr. F. K. Sparrow, Jr., and Dr. F. T. Wolf for their generous cooperation in sending cultures, and to Dr. B. B. Kanouse, Dr. F. T. Wolf, Mr. S. B. Salvin, Dr. K. B. Raper, Dr. J. V. Harvey, and Dr. A. G. Kevorkian for permission to include here records of their hitherto unpublished isolations of *Allomyces*.

LIST OF ISOLATES

Key to abbreviations, etc.:

The date immediately following the isolate name indicates the approximate time of isolation.

Isol. means "isolated by" and designates strains in the writer's collection which were isolated by other workers.

Rep. means "reported by."

Coll. means "collected by" and designates those people who made collections of soil from which the writer's isolates were obtained.

* designates strains of *A. arbusculus* isolated and named in the literature previous to the published description of sexuality in that species (cf., text pp. 108–109).

EUALLOMYCES

A. arbusculus

North America, U. S. A.

*Illinois: 1912, garden soil, Urbana. Rep. Barrett (1912).

*Kentucky: Spring 1928, soils, Richmond. 11 isolates, rep. Harvey (1930).

Louisiana: Oct. 1935, soil, New Orleans.

*Michigan: Soils. (a) Oct. 1930, edge of Pittsfield Pond, Ypsilanti. (b) Nov. 1930, flowerbed, Ann Arbor. (c) Nov. 1930, alfalfa field, Mason. Rep. B. B. Kanouse (unpublished notes).

*Mississippi: Spring 1927, soils. 27 isolates, rep. Harvey (1930). Some of these strains studied by Lugg (1929).

*New York: (a) 1912, soil and debris, bottom of inland lake near Ithaca. Studied by Barrett (1912). (b) Late winter and spring 1926, soils, near Cold Spring Harbor, Long Island. Several isolates, rep. Couch (1927). (c) Summer 1927, soils, Lockport. 2 isolates, rep. Harvey (1930).

North Carolina 1: 1926, soil under grass, Chapel Hill. Isol. W. C. Coker. Studied by Cotner (1930b), Sörgel (1937). To W. H. Weston from B. B. Kanouse, March 1934.

North Carolina 2: July 1931, stream near Raleigh. Isol. A. B. Couch. Studied by Hatch (1933, 1935, 1938). To W. H. Weston from W. R. Hatch, Dec. 1933. (Must arbitrarily be considered the type isolate of the species as regards the sexual stage. See p. 109).

*North Carolina: Other frequent isolations some of which are: (a) Oct. 1921, partly submerged knuckle bone of beef, Sparrow's Pasture, Chapel Hill. Studied by Coker and Grant (1922). (b) Feb. 1925, soil, three inches deep, under hemlock, and frozen loam six inches deep, Chapel Hill. 2 isolates, rep. Harvey (1925). (c) July 1926, soils, Springdale Farm, Haywood County. 2 isolates, rep. Coker and Braxton (1926). (d) 1927, soils, Chapel Hill, Charlotte, and Jones County. 9 isolates, rep.Coker (1927). (e) 1928, soils, Chapel Hill. 10 isolates, rep. Raper (1928).

North Carolina: 1939, water, near Burgaw. Rep. Ward, (1939).

*Oklahoma: Winter 1926–27, soil, Shawnee? Rep. Harvey (1930).

*South Carolina: Rep. Coker (1937), no details given.

Tennessee: soil, Nashville. To be reported elsewhere by F. T. Wolf.

Texas 1: Nov. 1934, soil, dried up ditch, near Stowell. Coll. R. H. Goodwin.

*Virginia: Spring 1930, field soils, Arlington. 2 isolates, rep. K. B. Raper (unpublished notes).

*Wisconsin: Summer 1926, soils, Madison. 3 isolates, rep. Harvey (1928).

North America, outside U. S. A.

Mexico 26 and 29: Autumn 1937, soils, Borda Gardens, Cuernavaca. Isol. and studied by Wolf (1939). To R. E. from F. T. Wolf Dec. 1937.

Mexico 37: Autumn 1937, soil, roadside ditch, near Tepexpan, 6 Km. east of Venta de Carpio. Other data as above.

Guatemala: Dec. 1938, soils. (a) Marshy area, Quirigua, altitude 250 feet. (b) Lake Calderas, altitude 7,000 feet. (c) Permanent marshy area, Guatemala City, altitude 7,000 feet. Rep. S. B. Salvin (unpublished notes).

Costa Rica: Soils. To be reported elsewhere by F. T. Wolf.

West Indies

Dominican Republic and Haiti: Soils. As above.

South America

Brazil 1, 2 and 5: Dec. 1938, soils, near Santa Cruz, Rio Grande do Sul. Isol. F. T. Wolf. To R. E. from F. T. Wolf, Jan. 1939.

Argentina: Soils, Misiones. To be reported elsewhere by F. T. Wolf.

Europe

Portugal 1E: Aug. 1939, granitic soil, pond, Montedor, Viano do Castelo. Coll. A. Burges.

Africa

Belgian Congo 1: Oct. 1934, soil, swamp bordering Lomami River, near Mukungu Village, North Katanga. Coll. J. Sandground.

Belgian Congo 2: Jan. 1936, soil, swamp near Lubilash River, Mulubule, Pania Mutombo, Kasai Distr. Coll. J. Bequaert.

Nyasaland 1: April 1938, soil, tropical rain forest, river valley near Mlanje. Coll. R. Leach.

Uganda 2: Aug. 1938, soil, bed of drain frequently filled with water, Kampala. Coll. C. G. Hansford.

Uganda 9: Aug. 1938, soil, shaded region under trees, Kampala. Coll. C. G. Hansford.
South Africa: Soils, Cape Province. To be reported elsewhere by F. T. Wolf.

India, Ceylon and Burma

*India 1: (a) still water, Pusa, Darbhanga, Bihar. (The type isolate of the genus and species.) (b) River water, Poona, Bombay. Studied by Butler (1911).

India 2: Jan. 1935, soil, bank of pond, City Park, Calcutta. Coll. E. Farell.

Ceylon 1: Nov. 1933, soil, near Dixwela, Nuwara Elija, altitude 6,200 feet. Isol. A. G. Kevorkian. Coll. P. French.

The following four isolates: Soils from ponds in the vicinity of Rangoon, Burma. Coll. G. W. Asher. Sent by E. E. Whittier.

Burma 1A: Oct. 1935, West Insein.
Burma 1C: Sep. 1935, Transport Road.
Burma 1Db: Sep. 1935, Lowis Road.
Burma 1F: Sep. 1935, North Kamogat Rifle Range Road.

China

China 2B: Oct. 1935, soil, ditch, Wuchang, Hupeh. Coll. H. H. Chung.
China 2G: Jan. 1936, soil, permanent pond. Other data as above.
China: Soils, Chengtu. To be reported elsewhere by F. T. Wolf.

Philippine Islands

Philippine Islands 1: June 1918, moss and adhering soil, drainage gutter, Los Baños. Isol. and studied by Weston (unpublished notes 1918–30).

*Manila: 1918–19, in algal cultures, The University, Manila. Rep. Weston (unpublished notes).

Malay Archipelago

Bali 1: 1929?, soil. Isol. and studied by Kniep (1930); studied by Sörgel (1936, 1937). To the Centraal-bureau voor Schimmelcultures at Baarn, Holland, from K. Noack; to F. T. Brooks from the C. B. S., Oct. 1937. (According to Sörgel, 1937, ff. p. 408, this strain came from the original material which Kniep obtained from Bali.)

Fiji Islands

The following six isolates: Aug. 1936. Soils, near Naduruloulou, Naitasiri Province, Fiji. Coll. courtesy of the Director of Agriculture Suva, 1936.

Fiji B1 and B2: Side of drain, dry land rice field, Central Agricultural Experiment Station.
Fiji C2: Side of deep drain, Central Agricultural Experiment Station.
Fiji F1 and F2: Roadside ditch where water constantly drips from bank of a cutting.
Fiji G2: Garden drain.

A. javanicus

North America

Arizona: Aug. 1937, sand, Totem Pole Wash, Monument Valley, Ariz., U. S. A., altitude 5,000 feet. To be reported elsewhere by J. V. Harvey.

Texas 2: Spring 1939, soil, bog, near Grapeland, Texas, U. S. A. Isol. F. K. Sparrow, Jr. Coll. G. R. LaRue. To R. E. from Sparrow, Aug. 1939.

Mexico 17: Autumn 1937, soil, Rio Pilon at intersection with C. N. 1, 833 Km. north of Mexico City. Isol. and studied by Wolf (1939). To R. E. from F. T. Wolf, Dec. 1937.

Africa

Tanganyika 3A: June 1938, soil, house drainage channel carrying intermittent water daily, Kiumba, Amani, Tanganyika Territory. Coll. H. H. Storey.

India and Burma

India B1, B3 and B4: Jan. 1937, soil, type locality where Butler (1911) collected *Allomyces*, Pusa, Darbhanga, Bihar. Coll. L. D. Galloway. (Note: B1 was lost before detailed measurements could be made.)

The following two isolates: soils from ponds in the vicinity of Rangoon, Burma. Coll. G. W. Asher. Sent by E. E. Whittier.

Burma 1Da: Sep. 1935, Lowis Road.

Burma 3: May 1935, near Shwe Dagon Pagoda. Isol. A. Howell.

Malay Archipelago

Java 1: 1929?, soil, Java. Isol. and studied by Kniep (1929, 1930). To W. H. Weston from Kniep, July 1930. (The type isolate of this species.)

Java: 1929?, soil, street gutter near Djokjakarta, Middle Java. Rep. Kniep (1930, p. 441).

Fiji Islands

Fiji D2 and D3: Aug. 1936, soils, damp hollow, banana plantation, Central Agricultural Experiment Station, Naduruloulou, Naitasiri Province. Coll. courtesy of the Director of Agriculture, Suva, 1936.

BRACHYALLOMYCES

A. anomalus

U. S. A.

Alabama: 1914, soil, ditch near Golf Course, Fairhope. Rep. and studied by Weston (unpublished notes 1914–26).

Mexico

Mexico 16: Autumn 1937, soil, Rio Pilon at intersection with C.N. 1, 833 Km. north of Mexico City. Isol. and studied by Wolf (1939). To R. E. from F. T. Wolf, Dec. 1937.

India

India B2: Jan. 1937, soil, type locality where Butler (1911) collected *Allomyces*, Pusa, Darbhanga, Bihar. Coll. L. D. Galloway.

CYSTOGENES

A. cystogenus

North America, U. S. A.

Arizona: March 1940, clay, Gila River, near Yuma. To be reported elsewhere by J. V. Harvey.

South America

Venezuela 1: Oct. 1935, soil, lagoon inland of Barrancas, at head of Orinoco River Delta. Coll. N. Weber.

Burma

Burma 1B: Sep. 1935, soil, drain, Judson College, Rangoon. Coll. G. W. Asher, sent by E. E. Whittier. (The type isolate of this species.)

China

China 2H: Jan. 1936, soil, temporary pool, Wuchang, Hupeh. Coll. H. H. Chung.

China 2J: Feb. 1936, soil, lotus field in shallow part of large lake, Wuchang, Hupeh. Coll. H. H. Chung.

A. moniliformis

U. S. A.

North Carolina: Soils. 4 isolates rep. Coker and Braxton (1926) and Coker (1927): (a) May 1926, moist sand five inches deep under *Hydrocotyle*, Smith Island. (The type isolate of this species). (b) and (c) July 1926, Pigeon River, Haywood County. (d) Vicinity of Smithfield.

North Carolina 3: Autumn 1934, soil?, Chapel Hill. Isol. J. R. Raper. To W. H. Weston from J. N. Couch, Dec. 1934.

Mexico

Mexico 46: Autumn 1937, soil, Rio Axtla at intersection with C.N. 1, 399 Km. north of Mexico City. Isol. and studied by Wolf (1939). To R. E. from F. T. Wolf Dec. 1937.

QUESTIONABLE

A. moniliformis?

Puerto Rico: Nov. 1938, on a cockroach, greenhouse sink, Agricultural Experiment Station, Mayaguez. Rep. A. G. Kevorkian (unpublished notes). (Unfortunately this culture was lost before conclusive identification could be made.)

A. sp.

U. S. A.

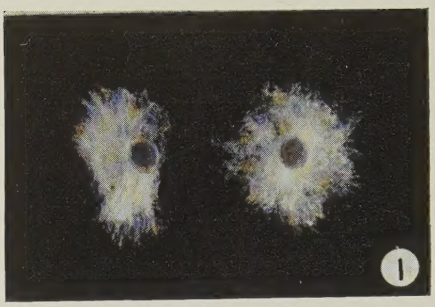
California: Feb. 1940, sand, dry wash near Palm Springs. To be reported elsewhere by J. V. Harvey.

METHODS OF ISOLATION AND CULTURE

It will be evident from an examination of the foregoing list that a large majority of the isolates were taken from soils, but it should be noted that most of the writer's own isolations were made from samples of earth or mud collected in places where water was or had been standing. In the writer's opinion *Allomyces* can fairly be called a truly aquatic fungus, as the most promising collecting sites appear to be ditches and drains or the edges of ponds, sluggish rivers, fresh-water swamps, rice fields and so forth where the banks are periodically wetted and dried. Of the soil samples collected for the writer from such localities in various parts of the world more than 25% contained viable resistant sporangia of *Allomyces*.

The 31 new strains which the writer isolated were all obtained in the following way. Quart mason jars, two-thirds full of water, were steam sterilized (15 lb. for 30 min.); when cooled each was inoculated with a few cc. of soil and "baited" with two hemp seeds, previously boiled for 15–20 minutes. Seeds of *Cannabis sativa* were used throughout and proved excellent as a standard substratum. Boiling served the dual purpose of sterilizing the seeds and splitting the hard, outer ovary-coat to allow penetration of the fungus. The inoculated jars were kept at room temperature, and when viable resistant sporangia of *Allomyces* (or spores of certain other fungi) were in the soil, a cottony growth of hyphae soon appeared on the seeds, often within two or three days (see Fig. 1). *Allomyces* developed rapidly and could usually be subcultured immediately, free of larger contaminants (Saprolegniales, Pythiales, etc.) by making transfers as soon as the first zoosporangia were formed, sometimes less than 48 hours after inoculation.

Standard Method of Water Culture.—Covered, glass crystallizing dishes (9 cm. diam. × 5 cm. deep) containing 150–200 cc. of water and one or two boiled hemp seeds were used to culture and study the fungus in what the writer somewhat arbitrarily considers a "standard natural environment." That this environment was "normal," or at least apparently highly favorable was indicated by the fact that under such conditions plants developed rapidly, were generally constant in form, and discharged large numbers of zoospores. Reduction in the amount of water or increase in dissolved nutrient, resulting from the use of too many hemp seeds, immediately produced abnormalities such as stunting or malformation of hyphae and sporangia,



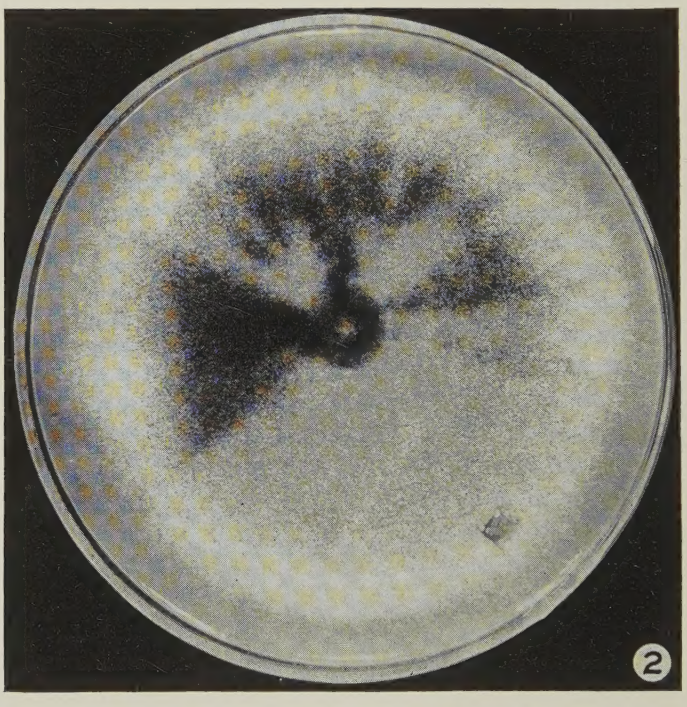


FIG. 1. A characteristic growth of *Allomyces* on hemp seeds in water culture. *A. javanicus*, asexual stage, natural size.

FIG. 2. A mixed gametophytic and sporophytic growth of *A. arbusculus* on agar, showing how noticeably the two stages differ in such cultures. The sporophytic sectors are darker because of the quantities of brown resistant sporangia borne on asexual hyphae.

formation of rhizoids on the fertile hyphae, nondischarge of zoospores, and so forth. Glass quart jars with 500–600 cc. of water and 3 hemp seeds were also used for water-cultures. The tap-water in Cambridge, Massachusetts when autoclaved, was found to be just as satisfactory as distilled water, but in Cambridge, England, a mixture of $\frac{1}{2}$ distilled and $\frac{1}{2}$ autoclaved, filtered pond-water was used. No attempt was made to keep the "bait" near the

surface of the water since growth as much as 8–10 cm. below appeared quite normal. All cultures were kept at room temperature (about 20–25°C.) unless otherwise indicated. Inoculation of water cultures was readily effected by transferring small tufts of fruiting hyphae or whole seeds with the attached fungus growth.

Pure Culture.—No special technique was necessary to obtain pure cultures. They could often be started merely by washing single hyphae through several changes of sterile water and placing them on nutrient agar, the surface of which was dry. If the growth was not free from bacteria at the start, one or two transfers, made by cutting out small blocks from the edge of the advancing mycelium, usually yielded a pure culture. When contaminants were particularly persistent, the use of very weakly nutrient agar checked bacterial growth and often appreciably increased the rate of extension of the fungus hyphae. It was necessary occasionally to resort to the usual method of plating out zoospores obtained by allowing a few zoosporangia or resistant sporangia to discharge in small petri dishes of sterile water.

Culture on Agar.—Allomyces was grown on agar by several earlier investigators (Barrett, Coker, Kniep, et al.). The writer has found that all of the species are very unrestricted in their nutrient requirements and will develop more or less vigorously on a wide variety of susbstrata such as yeast-starch, yeast-dextrose, oatmeal, sweet corn, hempseed, split pea, or peptone-levulose agars. The three following media were the most satisfactory however:

1. Oatmeal agar: 50 g. oatmeal in 700 cc. water, steamed for 1 hour, strained through muslin, and added to 20 g. of agar in sufficient water to bring the total volume to 1 liter.

2. Ye.S. agar: 100 cc. yeast autolysate, 15 g. corn starch, 1 g. K_2HPO_4, 0.5 g. $MgSO_4$, and 20 g. agar per liter of water.

3. Yp.Ss. agar: 4 g. powdered yeast extract (Difco), 15 g. soluble starch, 1 g. K_2HPO_4, 0.5 g. $MgSO_4$, and 20 g. agar per liter of water.

(The same sort of water found to be suitable for water cultures was used in agar media.)

Stock cultures were kept on slants of oatmeal agar or Ye.S. agar, in large test tubes (150–180 mm. long × 18–22 mm. diam.), and were usually transferred every six weeks at room temperature, although they could be kept viable for several months at 10°C. Yp.Ss. agar was very much more transparent than the other two media and was used, often at $\frac{1}{2}$ or $\frac{1}{3}$ strength, for most of the experimental work, i.e., isolation of single germlings, study of different stages in the life cycles, etc.

Great numbers of mature reproductive organs, resistant sporangia and zoosporangia on asexual mycelia and male and female gametangia on sexual mycelia, were formed in agar cultures at or just beneath the agar surface. Masses of them could readily be removed, uninjured, by gently scrap-

ing the top growth with a sterile needle and would, if immediately suspended in water, usually discharge swarmers in great profusion within a few hours.

ASEXUAL AND SEXUAL MYCELIA OF *Euallomyces*

Plants bearing sporangia always developed in water cultures inoculated in this manner with gametangia or zoosporangia from young, active mycelia. It was rather more difficult to obtain sexual plants of *Euallomyces* however. Since sexual mycelia are normally formed only by resistant-sporangial zoospores, to produce a pure sexual growth resistant sporangia had to be matured and separated from viable zoosporangia before they could be used as inoculum. The separation was easily effected indirectly by drying the resistant sporangia on filter paper and heating them until the less hardy accompanying zoosporangia and portions of asexual hyphae had been killed. A dry heat of 30–35°C. for a few weeks or 50–60°C. for some hours was usually effective. Healthy asexual material of a majority of the isolates of *Euallomyces*, derived either from water or agar cultures and bearing mature resistant sporangia, if pretreated in this manner would usually produce sexual plants when subsequently placed in water. Sterile resistant sporangia of all species of *Allomyces* were dried on strips of filter paper and put away in small, sterile vials; they could be used at any time thereafter, over a period of years (cf. p. 92), to start new pure cultures. The question of maturation is not well understood at present, but it should be pointed out here that resistant sporangia taken from young cultures would not ordinarily germinate without previously being somewhat aged and (or) dried, heated, frozen, etc. The writer found, however, that they would sometimes germinate when less than two weeks old even though kept continuously in water culture at an even temperature. An investigation of the controlling factors involved in "maturation" would be very worthwhile.

Obviously, water cultures of sexual plants of *Euallomyces* soon partially reverted to asexual as a result of gamete discharge and zygote formation. Hence, to keep unmixed gametophytic or sporophytic growths on agar the surface of the cultures had to be kept dry at all times. Agar slants and plates were allowed to dry for several days before being inoculated; cultures were kept at room temperature to prevent moisture condensation resulting from sudden temperature changes; frequent transfers were made (every 3–6 weeks) to ensure continued vitality and vegetative growth of the mycelia. Fig. 2 illustrates the effect of making transfers to moist-surface agar; this culture, though originally inoculated with sexual hyphae, became mixed as a result of gamete discharge and zygote formation with consequent appearance of asexual plants. Such reversals were usually easily detected, for sexual growth was colored bright orange by male gametangia, whereas asexual growth was colored dark brown by resistant sporangia.

Hanging-drop Cultures.—Hanging-drop mounts were ideal for isolating

spores for life-history studies and for observing spore germination, gamete fusion, early growth stages, etc. They were usually made up with sterile water "baited" with very small bits of hemp seed. Agar smears or drops of liquid nutrient media were also sometimes used for special purposes.

Terminology

There are in the genus *Allomyces* some four or five types of reproductive "organs," differing not only in origin and morphology but in the structure or subsequent behavior of the motile entities which they release. In order to avoid confusion the writer has consistently used certain terms as they are defined here:

Swarmer.—Any motile, flagellate, reproductive entity, either spore or gamete.
Zoosporangium.—The ordinary, thin-walled sporangium, borne on asexual plants of *Euallomyces* and all plants of *Brachyallomyces* and *Cystogenes.*
Zoospore.—The swarmer from a zoosporangium.
Resistant Sporangium.—The heavy-walled, brown, pitted sporangium, borne on asexual and sometimes on sexual plants of *Euallomyces* and on all plants of *Brachyallomyces* and *Cystogenes.*
R. S. zoospore.—The swarmer from a resistant sporangium.
Sporophyte.—The plant of *Euallomyces* which bears zoosporangia and resistant sporangia.
Gametophyte.—The plant of *Euallomyces* which bears gametangia and also, sometimes, resistant sporangia as well.

It is imprtant to distinguish sharply between the ordinary *zoospores* and *R.S. zoospores.* The writer is well aware that technical terms to designate the resistant sporangia and R.S. zoospores would be much more satisfactory. He believes, however, that it will be well to postpone giving new names to these and other structures in *Allomyces* until our knowledge concerning certain phases of their development and significance, particularly as regards the chromosome number of their nuclei, is more complete.

Characters of General Importance

Before describing the life cycles of *Allomyces* and discussing the morphological features which distinguish the various subgenera, species and varieties, it will be well to consider certain characters which are not of taxonomic value within the genus but are of fundamental importance in determining intergeneric and other broader relationships, for they show clearly the close similarities between the four genera of the Blastocladiales and the similarities and differences between this and other orders of the Phycomycetes.

MORPHOLOGY OF THE SWARMERS

The position and number of flagella and the structure of the swarmers of the aquatic Phycomycetes are of great significance in arranging these fungi in natural groups, so it is important to understand clearly here the character of the motile reproductive cells in *Allomyces* and the closely related genera. There has been, in the past, much controversy as to whether the

swarmers of the Blastocladiales are typically monoflagellate or biflagellate. By a series of detailed and careful studies Cotner (1930a and b) finally showed conclusively that almost all zoospores of *Blastocladia* and *Allomyces*, formed and discharged under optimum conditions, particularly of temperature, had but one flagellum; multiflagellate spores resulted from incomplete cleavage in the sporangium caused by unfavorable environmental factors. Cotner's findings were in agreement with those of Kniep (1929) who discovered a good many biflagellate and some triflagellate zoospores in *A. javanicus* but showed cytologically that in every case each flagellum had a corresponding nucleus, i.e., 2-flagellate swarmers were binucleate and so forth. Hatch (1938) reported that some of the biflagellate gametes which he occasionally found in *A. arbusculus* were uninucleate, but he emphasized the fact that a great majority of both the male and the female gametes had but one flagellum.

From the work of Matthews (1937) and Harder and Sörgel (1938 and 1939) on *Blastocladiella* and of Stüben (1939) on *Sphaerocladia* it is evident that in these two genera also the swarmers are typically monoflagellate. The writer's own studies of living material of *Blastocladia*, *Blastocladiella* and *Allomyces* have convinced him that zoospores, R.S. zoospores, and gametes throughout the order are characterized by a single, posterior flagellum, although 2-, 3-, or even 4-flagellate, oversize swarmers resulting from incomplete cleavage can often be seen. The only exception to this general rule is found in *Allomyces* subgen. *Cystogenes* where a majority of the primary R.S. zoospores are biflagellate, but there is some evidence to indicate that these structures result from sexual fusion and really correspond, therefore, to the biflagellate planozygotes of *Euallomyces* and other forms. (A discussion of this point will be found on p. 111.)

Another feature of interest and importance in the morphology of the swarmers is the large cytoplasmic inclusion which has been variously termed the "food-body" or "nuclear cap." Thaxter (1896) described a subtriangular body in the zoospores of *Blastocladia* but incorrectly interpreted the whole structure as a large nucleus of peculiar shape. Barrett (1912) first showed that the true nucleus is very much smaller and is imbedded in the posteriorly directed apex of an extranuclear cytoplasmic inclusion which he called the "food-body." The cytological investigations of Kniep (1929) and Hatch (1935) have verified Barrett's interpretation and shown that an exactly similar structure occurs in the gametes of *Allomyces*. It is also a very regular and constant feature in the swarmers of *Blastocladiella* (*Matthews* 1937, Harder and Sörgel 1938) and *Sphaerocladia* (Stüben 1939), and its presence clearly constitutes a character of ordinal importance in the Blastocladiales. Stüben has shown that there is a second inclusion, smaller and of different shape, situated adjacent to the large central one in *Sphaerocladia*.

From his extensive cytological studies of gametogenesis in *Allomyces arbusculus* Hatch (1935) concluded that the nuclear cap arises by a condensation of chondriosomal material. Both Kniep (1929) and Hatch (1938) have noted that when syngamy occurs in *Allomyces* the two nuclear caps of the gametes fuse some time prior to nuclear fusion. Hatch (1938) has also pointed out that one of the initial steps in zygote germination is the dissociation of the nuclear-cap material previously contributed by both gametes. The writer has found that, as one would expect, this dissociation of the food body also occurs in the very early stages of germination of zoospores and R.S. zoospores. Indeed the presence of the numerous granules resulting from partial disintegration of the nuclear cap is a confusing factor in nuclear studies of young germlings, for the "food-material" stains very deeply with most of the usual nuclear stains.

At the present time we are almost entirely ignorant as to the exact composition and function of the nuclear cap in the Blastocladiales, but Barrett's suggestion that it is a reserve food-material seems quite possible since it always disintegrates during the process of zygote- or spore-germination. Hatch (1935) has likened it to the limosphere of certain mosses and made comparisons with the nebenkern of Diptera.

(For figures and further details concerning the structure, origin and function of the nuclear cap in *Allomyces* see papers of Kniep and Hatch cited above.)

CHARACTER OF THE CELL WALLS

It does not seem pertinent to present a detailed summary of the studies of other investigators on the chemical composition of the cell walls in the Blastocladiales. The character of the wall has played such a considerable part in most of the discussions of relations in the Phycomycetes however, that it should be pointed out here that nearly all of the investigations which have been made indicate that all true blastocladiaceous fungi have cell walls which do not react positively to any of the tests for cellulose. On the contrary the walls in this group, in contrast to those of most other oomycetes, are apparently composed of substances akin to chitin. In a recent paper on the membrane of lower fungi Nabel (1939) reviews the literature on this subject and reports positive tests for chitin in *Allomyces*, *Blastocladiella*, and *Sphaerocladia*. (See also Kanouse 1927, and Harder 1937.)

MORPHOLOGY AND SIGNIFICANCE OF THE RESISTANT SPORANGIA

The heavy-walled structures which have been called "resting cells," "resting spores," "Dauerzellen," "Dauersporangien," or resistant sporangia by the various investigators are very characteristic of all the true Blastocladiales. They are formed within the original hyphal membrane but are not fused to it and have a two-layered wall of their own, the

outer of which is usually thickened and brown. In *Blastocladia* and *Allomyces* this outer wall is almost always sculptured with fine pits in a very characteristic manner (cf., Figs. 12–15). Barrett (1912) believed that the markings were actual pores passing right through the wall, but Thaxter (1896), Coker and Grant (1922), Coker (1923) and others have correctly figured or described them as pits. In *Blastocladiella* the sculpturing is rather netlike, giving an appearance of irregular shallow pits, while in *Sphaerocladia* the wall is smooth.

The assumption or conjecture that resistant sporangia in the Blastocladiales are oospores, usually parthenogenetically formed, was emphasized by many of those who studied the group before 1929. Kanouse (1927) strongly favored this view and figured occasional, coiled, so-called "antheridial" filaments on two plants of *Blastocladia globosa*. No relation between these filaments and the resistant cells was shown however, and fertilization was never observed. Kniep's discovery of the sexual stage and the true significance of resistant sporangia in *Allomyces* exploded the "parthenospore" theory for that genus and immediately cast serious doubt upon its validity for *Blastocladia*. The discoveries by Harder and Sörgel (1938) and Stüben (1939) of isoplanogametes in *Blastocladiella* and *Sphaerocladia* made it quite clear that resistant sporangia of these forms correspond exactly with those of *Allomyces* in origin and function. Resistant sporangia of all four genera are essentially similar in structure and in behavior at germination. In view of this similarity and of the lack of any conclusive evidence to the contrary therefore, resistant sporangia of *Blastocladia* must no longer be considered oospores or parthenospores, even though we do not yet know exactly what sort of sexual reproduction, if any, occurs in this genus. Throughout the Blastocladiales the resistant sporangia are, as their name implies, asexually formed resistant structures which liberate swarmspores. They may be considered as a logical corollary to the zygotes which do not develop into resistant structures in this group, as they usually do in other Phycomycetes, but germinate immediately to form new plants. The resistant sporangia, therefore, serve to carry the blastocladiaceous fungi through periods of adverse environmental conditions. In *Allomyces* they are extraordinarily resistant; the writer has found that, when thoroughly dry they can remain viable for several hours at 100°C. or a number of years at ordinary temperatures. W. H. Weston has reported (unpublished notes) that resistant sporangia of his isolate from Alabama were still capable of germination after being dried for as long as 10 years.

DEVELOPMENT AND MORPHOLOGY OF THE THALLUS OF ALLOMYCES

The vegetative thallus of *Allomyces* is very characteristic and almost exactly the same in all the known species. It develops from zygotes or from

swarmers, always, as shown in Fig. 3, in the following manner: the first germ tube is slender and ultimately forms the system of tapering rhizoids, while the second, heavy tube, protruding somewhat later from the region opposite the place of origin of the rhizoids, develops into the main hyphal part of the thallus. Under favorable conditions, at room temperature, a mature thallus will develop from a spore in 36 to 48 hours, and the regu-

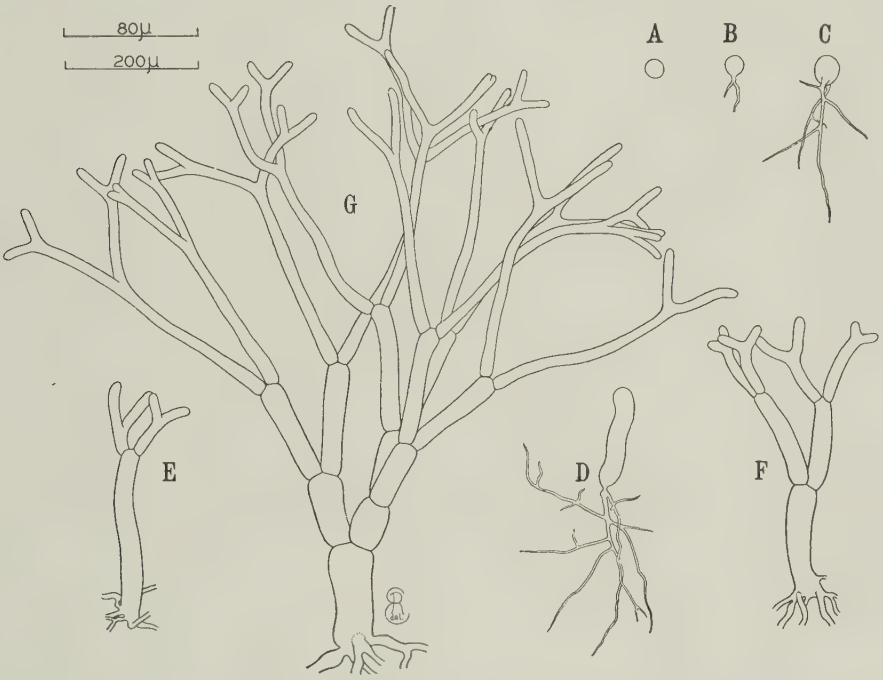


FIG. 3. Stages in the germination and development of a zoospore: A, quiescent and rounded spore about to germinate; B and C, young germlings with slender, tapering, rhizoidal germ tubes; D, older germling with rhizoidal system and stout hyphal tube; E and F, young plants; G, nearly mature thallus, showing the characteristically regular dichotomy. Upper scale for A–D, approximately × 220 as here reproduced; lower scale for E–G, approximately × 55 as here reproduced. Zygotes, zoospores and R.S. zoospores (except primary R.S. zoospores of *Cystogenes*) develop in this manner in all species of *Allomyces*.

larity of the dichotomy is striking. In water culture, growing on hemp seed, plants ordinarily extend out 1 to 1.5 cm. from the substrate, but their final size depends to a large extent on the amount of available nutrient and on other environmental factors. Under foul conditions the plants may be stunted and irregular in form; when they are crowded they tend to be more slender and develop less fully; when the supply of nutrient is very small plants which are only 40 or 50μ in height may form reproductive organs (cf. Fig. 8, f). Because of this variability and the similarity of the thallus of all the known species under comparable conditions the size of whole

plants or their component vegetative "cells" is not of taxonomic significance within the genus; it is only of value in distinguishing *Allomyces* from the three, markedly smaller, related genera.

In side view, as in Fig. 3, the hyphae of *Allomyces* appear septate, but close examination reveals that the cross walls are incomplete. The peculiar, false septations characteristic of this genus were described and figured in detail by Barrett (1912), who showed that they are ring-like thickenings, extending centripetally from the hyphal walls in younger portions of the thallus and later forming central plates with radiating strands. An excellent photomicrograph (taken by A. B. Couch) of one of these wheel-shaped pseudosepta is shown in a paper by Coker (1930, Pl. 9). Strictly speaking, the thallus of *Allomyces*, like that of *Blastocladia*, is unicellular.

Basis of Taxonomic Arrangement

We may turn now to a consideration of the criteria which the writer has used to distinguish the subgenera, species and varieties of *Allomyces* described in the present paper. A discussion and explanation of the various points of similarity and difference between the groups will, it is hoped, clearly establish the validity of classifying them according to the method used in the Taxonomic Outline (pp. 126–134) which follows.

1. Criteria for Establishing Subgenera

Inasmuch as there is very close similarity between the sporangium-bearing plants of all species, i.e., the asexual generation of *Euallomyces* and the only evident generation of *Brachyallomyces* and *Cystogenes*,—the life history must obviously be given special recognition in establishing subgenera, for, as the writer (1938 and 1939) has shown, three very different life cycles, summarized in the accompanying chart, are now known. Although these cycles are briefly described in the Taxonomic Outline it will be well to discuss them here in some detail noting certain points of particular interest.

Subgenus A. *Euallomyces*

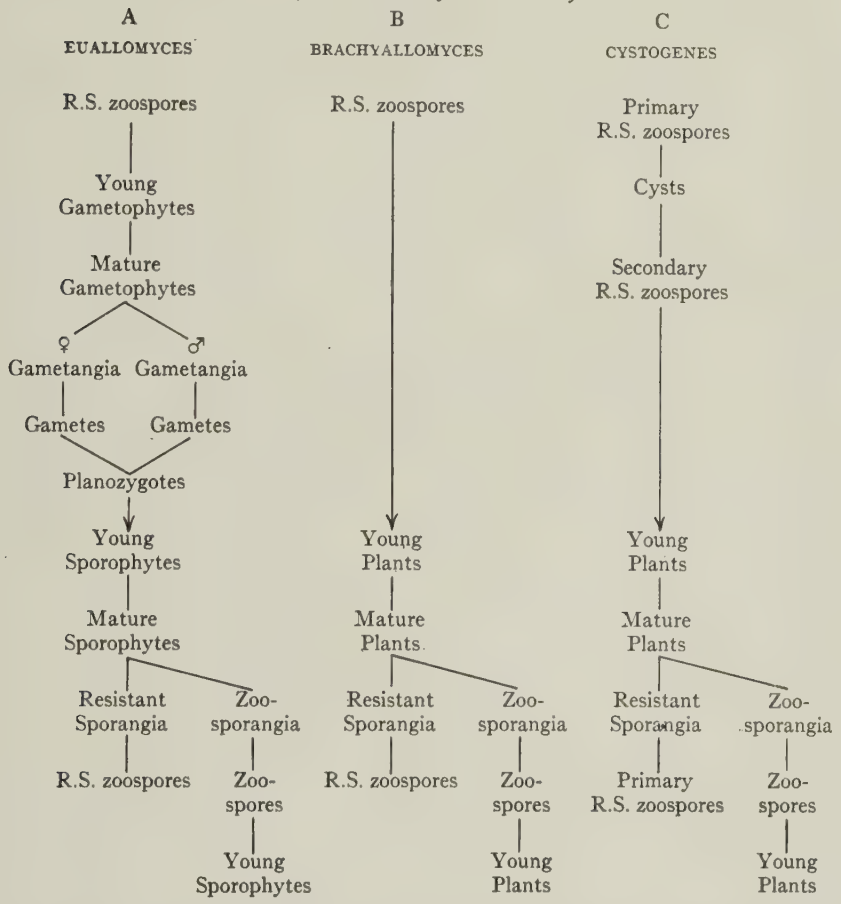
NORMAL LIFE CYCLE AND THE QUESTION OF NUCLEAR BEHAVIOR

As shown in Fig. 4 this cycle is initiated by germination of the resistant sporangia and discharge of the R. S. zoospores which, after a more or less extended swarm-period, settle down, round up, lose their flagellum and germinate with a tube in precisely the same manner as the swarmers from thin-walled zoosporangia (compare C and M, Fig. 4). The young gametophyte plants, until they start fruiting, are indistinguishable from young sporophytes and both grow and develop in the same way (cf. Fig. 3). After 24–48 hours, however, gametophytic hyphae (E, Fig. 4) start to subtend male and female gametangia. These reproductive organs correspond to

zoosporangia in their mode of formation and general arrangement. The females are colorless or gray and also similar to asexual sporangia in size, but the males can soon be readily distinguished by their striking, orange to

CHART

Summary of the Life Cycles in *Allomyces*



salmon or brick-red color. Plants are hermaphroditic and self fertile,* and primary gametangia (those formed terminally) usually occur in pairs, a male and a female together, arising at the same time and discharging gametes nearly simultaneously (F, Fig. 4).

* Possibly when Coker (1937, p. 1) stated that *Allomyces* is heterothallic he meant to indicate that there are two sorts of thalli asexual and sexual. This use of the term heterothallic, however, is entirely contrary to its original and accepted meaning, i.e., the condition in which two sexual strains are segregated in separate thalli. Hence it should be noted that *Allomyces* is *not* heterothallic, but is, on the contrary, homothallic or hermaphroditic.

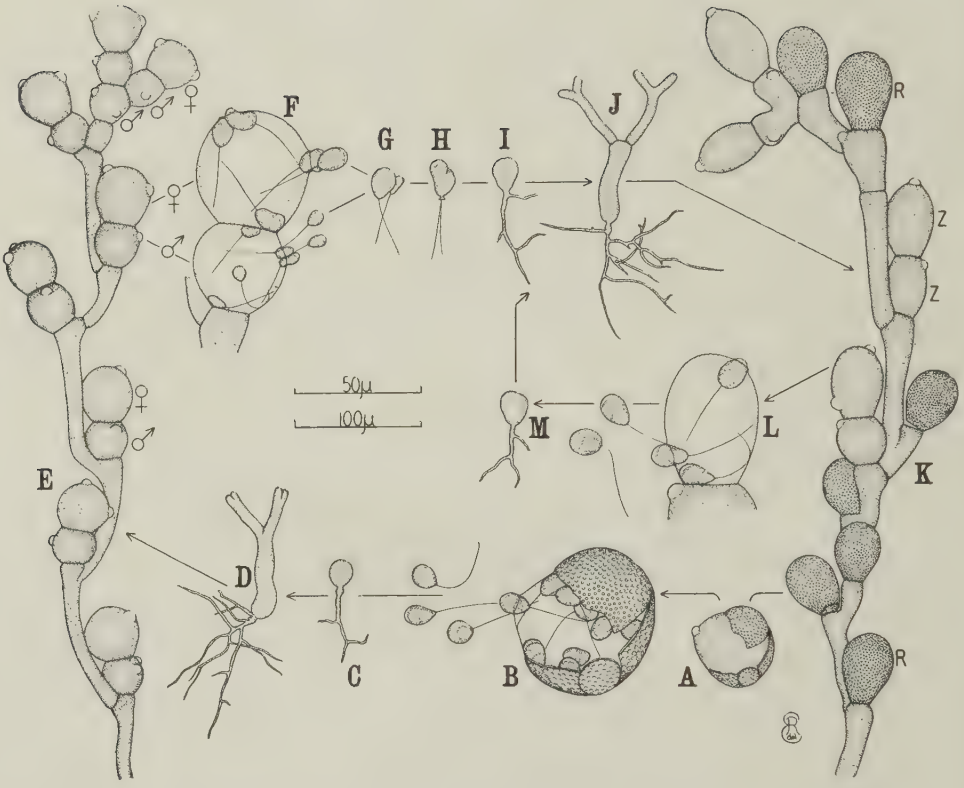


FIG. 4. The life cycle of *Euallomyces*, illustrated here with *A. arbusculus;* A, germinating resistant sporangium with outer wall split and a papilla of discharge already formed on the inner membrane; B, R.S. zoospores emerging from the germinated resistant sporangium; C, germinating R.S. zoospore; D, young gametophytic plant; E, mature gametophytic hypha bearing ♀ and ♂ gametangia; F, ♀ and ♂ gametes emerging from a pair of gametangia; G, copulating gametes; H, planozygote; I, germinating zygote; J, young sporophytic plant; K, mature sporophytic hypha bearing thin-walled zoosporangia (Z, Z) and heavy-walled resistant sporangia (R, R); L, zoospores emerging from a zoosporangium; M, germinating zoospore. Upper scale for B, C, F–I, L and M, approximately × 335 as here reproduced; lower scale for A, D, E, J and K, approximately × 165 as here reproduced.

Female gametes are about the same size as zoospores or slightly smaller, but they do not normally develop without being fertilized (see p. 99 for discussion of parthenogenetic development). Male gametes, tinged a pale brassy color by the pigment which is apparently contained in their lipoid granules, are strikingly smaller than females, usually about one-half the diameter, and rather more active. Both have the same basic structure described above (p. 89). In his papers on gametogenesis and conjugation in *Allomyces arbusculus*, Hatch (1935) has described in great detail the origin and structure of the gametes. Copulation is difficult to follow through all stages in living material and sometimes takes place almost instantaneously.

Plasmogamy is often preceded by a longer or shorter period in which the paired and closely appressed gametes (G, Fig. 4) exhibit striking semi-amoeboid activity. From fixed and stained material, Kniep (1929) and Hatch (1938) have figured and described in detail the various fundamental stages in the process of fusion.

The biflagellate planozygotes (H, Fig. 4) are sluggishly motile for a short period, usually not more than 5 or 10 minutes, and soon settle down, round up and germinate directly by a germ tube (I, Fig. 4) without any period of rest. The young sporophyte germlings (J) develop into mature plants (K) bearing zoosporangia (Z, Z) and resistant sporangia (R, R). Zoospores from zoosporangia reproduce the sporophyte asexually (L and M, Fig. 4) just as they do in *Brachyallomyces* and *Cystogenes*. R. S. zoospores, on the other hand, from resistant sporangia of *Euallomyces*, again give rise to gametophytic plants thus completing the cycle.

Germination of resistant sporangia of all forms takes place as shown in A and B, Fig. 4. Cracking of the outer wall is evidently a result of pressure from within, and the content, surrounded by a thin inner membrane, may continue to swell until it becomes more than twice its original diameter. One to four or occasionally more discharge papillae are formed, exactly similar to those on the zoosporangia and gametangia. Germination, when once initiated, takes place within a short period, often less than sixty minutes after splits first appear in the outer wall. There are indications that these splits occur along definite predetermined lines (Sörgel, 1937, Fig. 7, p. 421), but the writer has found that it is frequently impossible to discern the lines clearly before germination has begun. After most of the spores have emerged the thick elastic outer wall of the sporangium frequently closes in again almost regaining the position which it originally occupied at the start of germination. In so doing it may crumple the thin inner membrane and trap a few of the spores still remaining within.

Once, in a water culture which was heavily contaminated with bacteria, the writer found a few resistant sporangia of *A. arbusculus* behaving as though they were conidia and germinating by means of thick hyphal tubes. Sporangia were formed at the ends of these hyphae and swarmers were discharged, but unfortunately no observations were made concerning their nature or subsequent behavior. Ordinarily, however, resistant sporangia of all species of *Allomyces* discharge swarmers directly, in the manner described just above.

Up to this point no mention has been made of the behavior of the nuclei in the life history of *Euallomyces*, and it will be well to review our present knowledge briefly here. As in many of the fungi the nuclei in *Allomyces* are very small, and mitotic or meiotic figures are extraordinarily difficult to stain clearly. It is not surprising therefore, that cytological work on this form has been contradictory. From the 1:2.12 ratio which he found be-

tween the average volume of the nuclei in gametophytic and sporophytic hyphae respectively, Kniep (1930) concluded tht meiosis occurs in the resistant sporangia of *A. javanicus*. That is to say, he believed that there was an antithetic alternation of haploid sexual plants (av. nuclear vol., 1) and diploid asexual plants (av. nuclear vol., 2). This belief was upheld by the studies which Sörgel (1936) made on *A. arbusculus* (*Kniepii*); Sörgel (loc. cit. ff. p. 1) stated that there were 6 chromosomes in the gametophyte and 12 in the sporophyte of this species, but details of the work were not reported. In an extensive study of nuclei in normal plants as well as those which were apparently heteroploid, Sörgel (1936) always found, furthermore, a close 1:2 ratio between the average volumes of nuclei in sexual and asexual hyphae respectively. His subsequent investigations (1937) of the alternation of generations in another strain of *A. arbusculus* were in agreement with this earlier work. Contrary to the findings of Kniep and Sörgel, however, Hatch (1938) concluded from a study of nuclei in zygotes of *A. arbusculus* that meiotic divisions occur at the time of zygote germination, i.e., that sporophytic as well as gametophytic plants are haploid, with 6 chromosomes, while the zygote represents the only diploid part of the cycle, with 12 chromosomes.

It seems clear from these conflicting reports that chromosome studies in *Allomyces* are open to individual interpretation, doubtless owing to the small size of the chromosomes and the large amount of granular, cytoplasmic material which frequently stains deeply with many of the usual nuclear stains. Hoping that it might be possible by genetical methods to clear up the conflicting views regarding the location of the meiotic divisions, the writer has made a study of inheritance in interspecific hybrids of *Euallomyces*. This work is now being completed and will be reported in detail elsewhere, but it will be pertinent to outline the essential points here. Reciprocal crosses were made between *A. arbusculus*, in which the male gametangia are characteristically hypogynous (subterminal in the primary pairs) and *A. javanicus* var. *macrogynus*, in which the male gametangia are always epigynous (terminal in the primary pairs). The arrangement of the gametangia borne on sexual plants derived from the F_1 asexual generation was then studied, and the results, very briefly, are as follows: (1) Gametophyte plants arising from any single F_1 sporophyte (obtained from a *single* zygote) are of many different sorts, i.e., both parental types, 100 per cent hypogynous and 100 per cent epigynous, segregate out, as well as a series of intermediates ranging from types which show nearly pure epigyny to those showing nearly pure hypogyny. (2) An exactly similar series of gametophytes can be obtained from any secondary F_1 sporophyte started from a *single zoospore* discharged from a zoosporangium borne on a primary F_1 sporophyte. These results indicate (a) that the arrangement of gametangia is a quantitative character controlled by polymeric genes, (b) that

meiosis does not occur in the zygotes of *Euallomyces*, and (c) that reduction divisions and segregation of the parental characters take place after the formation of resistant sporangia on the sporophyte and before the development of mature gametophytes. Hence, although the final interpretation of this work must await the analysis of the F_2 generation, it seems probable that Kniep's original concept is correct and that there is in most, if not all strains of *Euallomyces* a regular alternation between diploid asexual and haploid sexual plants. As Kniep (1930) pointed out, this is a most unusual state of affairs: extensively developed thalli with true, actively dividing diploid nuclei are probably not ordinarily formed by any other Phycomycetes.

DEPARTURES FROM THE USUAL LIFE CYCLE

Before passing on to a discussion of the other two life cycles in *Allomyces* we must consider here some of the variations or deviations which are known to occur more or less frequently in the normal life history of *Euallomyces* which has just been reviewed.

Apomixis

By separating male and female gametangia Kniep (1929, 1930) first showed that female gametes of *A. javanicus* can occasionally develop, without fertilization, into mature plants. In Kniep's experiments these plants were always sexual and bore male as well as female gametangia. The writer too has been able to obtain mature gametophytic plants from female gametes of both varieties (*javanicus* and *macrogynus*) of *A. javanicus* (cf. Fig. 5). Sörgel (1937), in his extensive study of the departures from the usual life cycle in two strains of *A. arbusculus*, showed that here, as well, female gametes sometimes develop parthenogenetically into normal, mature sexual plants. He also made the significant discovery, however, that *parthenogenetic female gametes of A. arbusculus occasionally give rise directly to asexual plants*, and the writer has been able to verify this repeatedly with several different strains of the species. This is contrary to Kniep's belief that female gametes could be distinguished from asexual zoospores by the fact that the former, when unfertilized could give rise to sexual plants only, while the latter always produced asexual individuals. Although the whole question of parthenogenetic development is most interesting from the experimental point of view, under natural conditions, where male and female gametes are discharged in direct proximity, simultaneously, in large numbers, the chances that parthenogenesis will occur are almost negligible.

The writer is entirely in agreement with other investigators regarding the fact that male gametes of *Allomyces* can not apparently develop in any way other than by normal fusion with females to form zygotes. Male gametes in this genus have never been induced to develop without fusion (by ephebogenesis), nor have they ever been seen to fuse with each other.

Sporophytes from R. S. Zoospores

A departure from the typical cycle which does undoubtedly occur frequently in nature has also been reported by Sörgel (1937) who found that

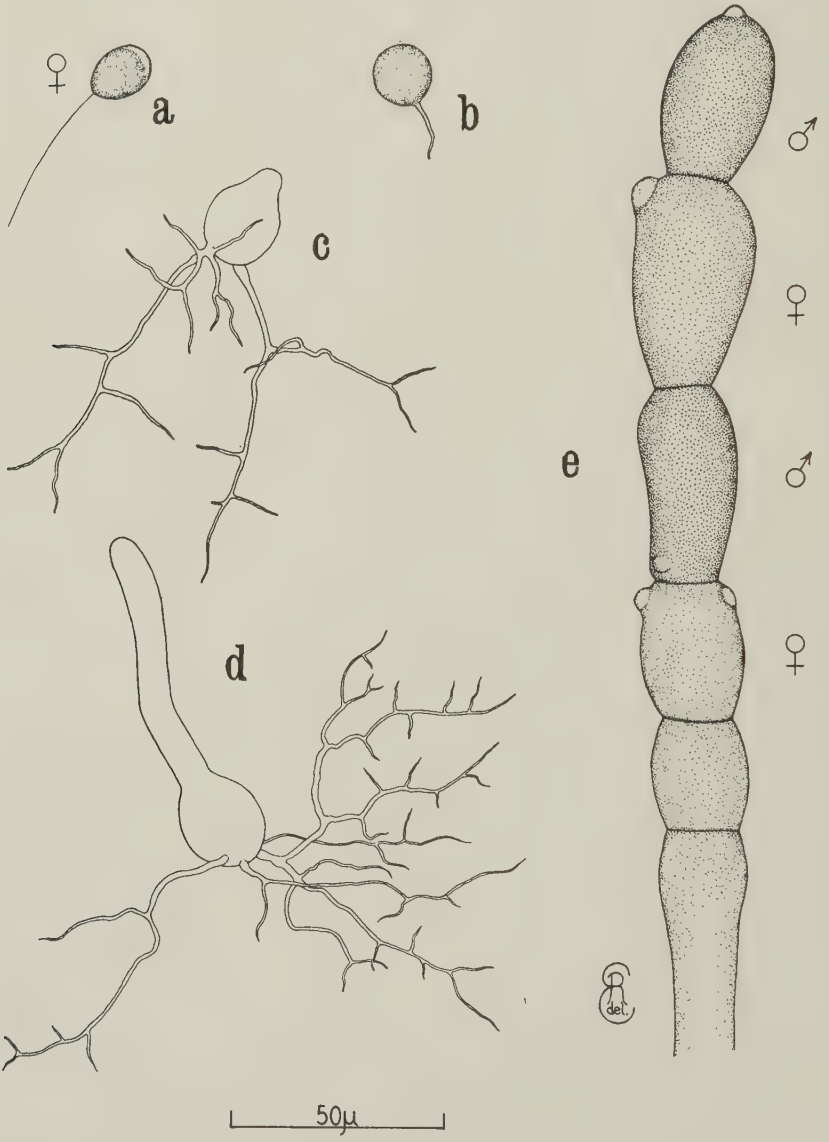


FIG. 5. Stages in the parthenogenetic development of female gametes of *A. javanicus* (isolate Java 1): a, motile female gamete; b, female gamete germinating parthenogenetically; c and d, parthenogenetic germlings; e, hypha of a mature parthenogenetic plant bearing both ♀ and ♂ gametangia. Approximately × 600 as here reproduced.

R. S. zoospores of *A. arbusculus* sometimes give rise directly to sporophytic rather than gametophytic plants. The writer has made a careful study of this phenomenon in a majority of the isolates in his collection. In some strains (see columns 2 and 3 of Table I) the R. S. zoospores gave rise to sporophytic plants so regularly that it was only after repeated germination of resistant sporangia under widely different conditions that sexual plants were finally obtained. The ordinary method which the writer has used to obtain gametophytic material has been described in detail above (see p. 88). With some isolates studied over a period of several years, after frequent failures to find any trace of hyphae bearing gametangia in water cultures inoculated with resistant sporangia, a careful search finally re-

TABLE 1. Showing the relative frequency and regularity with which different isolates of *Euallomyces* form sexual plants from R.S. zoospores.

1. Regularly form sexual plants in water-culture.	2. Do not regularly form sexual plants in water-culture.	3. Induced to form sexual plants only under special conditions.
A. arbusculus		
Mexico 29 and 37	North Carolina 1	Texas 1
Bali 1	China 2G	Mexico 26
Burma 1A and 1Db	Burma 1C	Fiji B1
North Carolina 2	India 2	
Philippine Isls. 1	Belgian Congo 2	
China 2B		
Ceylon 1		
and others		
A. javanicus		
Burma 3 and 1Da	Mexico 17	Fiji D3
India B4		Tanganyika 3A
Java 1		
and others		

vealed a very few scattered sexual plants hidden in the large sporophytic growths. In such cases, where there had been copious, rapid germination of resistant sporangia it is clear that a majority of the R. S. zoospores had given rise to sporophytic plants. With other isolates, in spite of painstaking search, no sexual plants at all could be found in water cultures. To test these forms a second method was resorted to: large numbers of resistant sporangia were scraped from rather dried up, 8 to 12 weeks old agar cultures of sporophytic material and placed directly on fresh, moist oatmeal agar plates. Several drops of sterile water were than placed on the inoculum, and since the agar was freshly prepared this water was not completely absorbed until 24–48 hours after the inoculation was made. Under these conditions a sporophytic growth often developed, either from viable portions of sporophytic mycelium in the inoculum or from zoospores discharged from thin-walled sporangia. In other cases however, there was immediate, mass germination of resistant sporangia, and sexual mycelia were

formed. Occasionally the cultures which developed were entirely gametophytic, or whole sectors of gametophytic growth appeared between adjacent sporophytic mycelium (as in Fig. 2). Sometimes, although superficial inspection showed that the cultures were an even brown color and apparently entirely asexual, a close examination of the hyphae under a dissecting microscope revealed small patches of mycelium bearing gametangia hidden among the sporophytic mass. It was usually possible in such cases to tease out tufts of the gametophytic material with needle-tweezers, transplant them to fresh agar and thus obtain pure sexual growths. The sexual stage of 5 particularly obdurate forms, which had never given sexual plants in ordinary water cultures, were obtained in this way. Only then was it possible to assign them to definite species: three of them (Texas 1, Mexico 26, and Fiji B1) proved to be *A. arbusculus* while the other two (Tanganyika 3A and Fiji 3) were *A. javanicus*.

Thus it is evident that we have a series of forms in either species of *Euallomyces*, morphologically closely similar but physiologically different, grading gradually from those, such as North Carolina 2 and Burma 3 (Column 1 in Table 1), which regularly produce a sexual stage, to those, such as Fiji B1 and Fiji D3 (Column 3), which apparently form sexual plants very rarely and only under rather special conditions. The three categories set up in Table 1 are for the purpose of roughly grouping the various isolates, but it must not be supposed that there is an absolute difference between them. From a careful statistical study it would probably be possible to arrange the isolates in a completely graded series.

Exactly what external conditions exert a controlling effect on the R. S. zoospores and determine whether they shall develop into sexual or asexual plants is not at all clear at present. Sörgel (1937) noted however, that when R. S. zoospores of *A. arbusculus* (*Kniepii*) germinated very soon after emergence from the sporangium they were more likely to produce gametophytes, whereas, after an extended swarm period they frequently formed sporophytes. This agrees closely with the writer's observations, for where resistant sporangia germinated on nutrient agar favorable conditions for germination were immediately at hand and R. S. zoospores probably swarmed for a very short period. In such cases the writer found that gametophytic hyphae were frequently produced. In water cultures on the other hand most of the R. S. zoospores had a more or less extended period of free swimming before they settled on the localized nutrient substratum, and under these conditions sporophytic plants of Mexico 26, Fiji B1 and similar isolates were always formed.

Further study will be necessary to elucidate this problem of the behavior of the R. S. zoospores as well as the closely related question, why do female gametes which develop parthenogenetically sometimes produce gametophytes and at other times develop into sporophytes? Possibly the double

capability of these swarmers (the female gametes and the R.S. zoospores) in *Allomyces* is comparable in some measure with the dual ability of the swarmers of *Olpidium Viciae* to behave either as zoospores or gametes depending, as Kusano (1912) discovered on environmental conditions.

It is logical to suppose that R.S. zoospores might give rise to sporophytic plants as a result of fusions with each other to form, as it were, premature zygotes such as are known to occur in certain algae. In the forms of *Euallomyces*, described just above, in which R.S. zoospores almost invariably produced asexual plants directly, detailed careful observations of spores from resistant sporangia were repeatedly made by the writer to ascertain whether such fusions did occur. Resistant sporangia were germinated time and time again in hanging drops with nutrient substrata where the swarmers could be studied under high magnification during their emergence, germination, and subsequent development into mature asexual plants, but *in no case were indications of fusion ever seen.* Sörgel (1937) reported two examples of apparent fusion between R.S. zoospores in an aberrant strain of *A. arbusculus*, but he admits that fusions of this sort were never seen in normal material. The writer is of the opinion that such fusions occur, if at all, only *very* rarely in *Euallomyces* (or *Brachyallomyces*, see below) and play no significant part in the normal life histories of these forms.

It is worth noting here that a similar possible explanation of the formation of sporophytes as a result of fusions between female gametes has been considered. But here again, from careful microscopic observations of many different lots of material the writer can state definitely that he has found no evidence whatsoever of the occurrence of such fusions.

Formation of Resistant Sporangia on Sexual Plants

Finally, we must consider a third and regularly recurrent departure from the simplified ideal life-cycle scheme which Kniep (1929, 1930) defined in *A. javanicus*. One of the reasons why Kniep differentiated the gametophyte and sporophyte so sharply was because he never observed resistant sporangia on the former but found them to be consistently characteristic of the latter. In April 1934 the writer discovered numerous resistant sporangia borne on sexual plants of *A. arbusculus* var. *minor* (Ceylon 1) as shown in Fig. 6. Since that time they have frequently been found, clearly produced on hyphae which were also bearing gametangia, in agar as well as older water cultures of a majority of the isolates of both *A. arbusculus* and *A. javanicus*. They have also sometimes appeared in particularly large numbers on sexual plants derived from interspecific hybrids to be described in detail in a forthcoming paper. It is clear therefore that the formation of resistant sporangia on sexual hyphae of Euallomyces occurs regularly under certain conditions.

Exactly how this state of affairs, the formation of sporangia on a game-

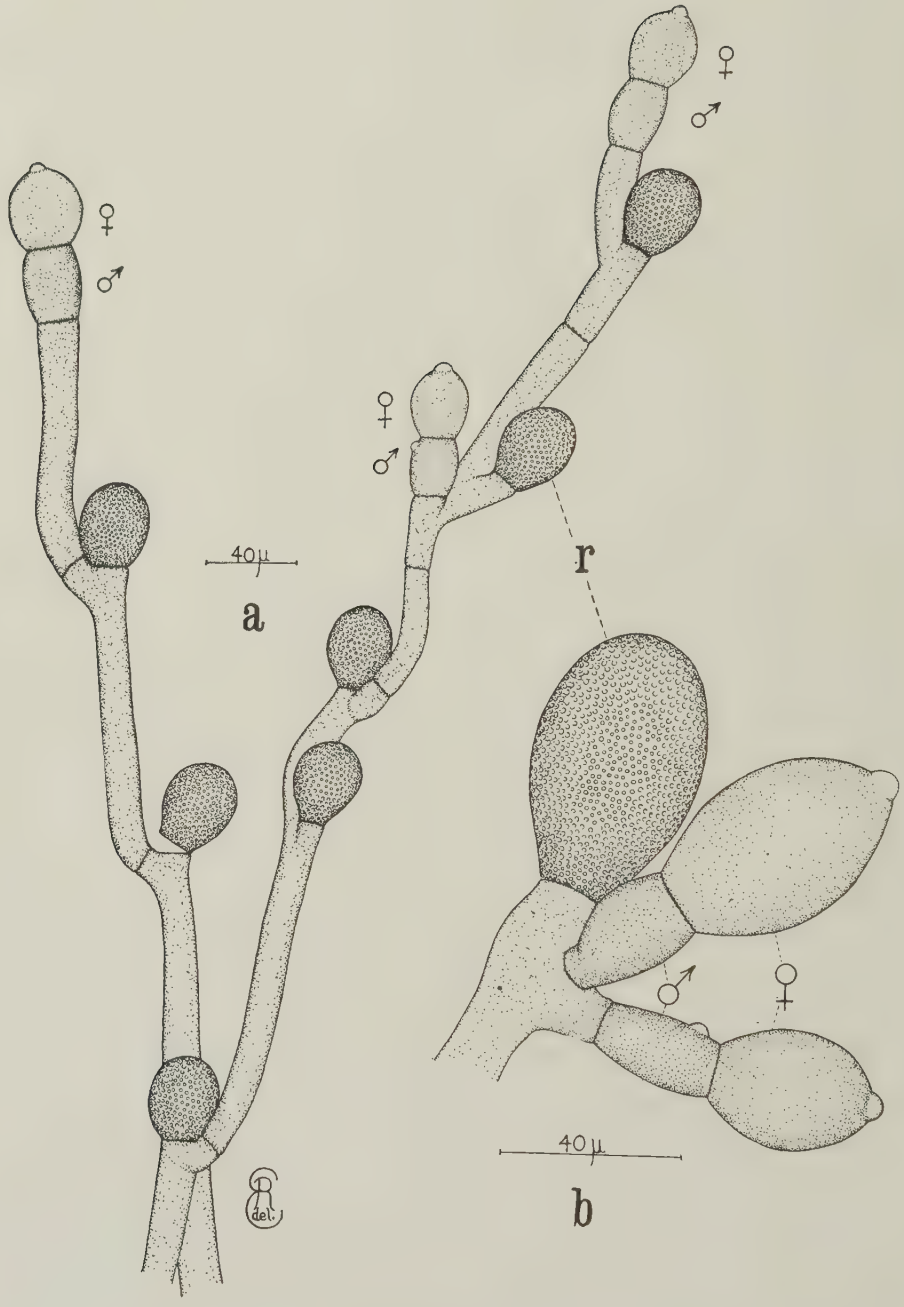


FIG. 6. Gametophytic hyphae of *A. arbusculus* bearing resistant sporangia (r) and ♀ and ♂ gametangia: a, var. *minor* (isolate Ceylon 1), approximately × 300 as here reproduced; b, var. *arbusculus* (isolate China 2B), approximately × 600 as here reproduced.

tophyte, can be interpreted is not precisely clear at present. Sörgel (1937) also observed the formation of resistant sporangia on hyphae bearing gametangia in the strains of *A. arbusculus* which he studied. He believes that this condition is correlated with a mixture of gametophytic and sporophytic nuclei within the same hyphae. He describes intergrades and direct change-overs from sexual to asexual plants and vice versa and gives some very interesting correlative measurements of nuclear volumes in such "mischhyphen" as he terms them. Chromosome studies were not made, however, and the acceptance of such an unusual condition, haploid and diploid nuclei within the same coenocyte, should perhaps be reserved until more detailed cytological investigations have been carried out. Furthermore it is not, in the writer's opinion, entirely valid to conclude, as Sörgel does, that in *Allomyces* the presence of sporangia in general indicates true sporophytic tendencies in a gametophyte. A sharp distinction must be made here between resistant sporangia and zoosporangia: that a mycelium is bearing resistant sporangia, which produce haploid spores, is no proof that it is also bearing the characteristic, asexual sporangia of the sporophyte, zoosporangia, which produce diploid spores. Zoosporangia are morphologically so similar in all ways to female gametangia, which are *not always* formed in direct association with male cells, that the occurrence of zoosporangia on sexual plants would be very difficult to prove. Female gametes growing parthenogenetically may, as we have seen, produce sporophyte plants just as zoospores do, but, as Kniep (1929) first pointed out, the female gametes develop very much more slowly than the zoospores. Comparative studies of germination and growth rate might possibly be used then in attempting to ascertain whether a reproductive organ were a sporophytic zoosporangium or a gametophytic female gametangium, but they would not be entirely conclusive. Sörgel, however, apparently did not undertake to make this necessary distinction; he does not make it at all clear on what criterion he based his decision that the two structures in Fig. 8, p. 425 (1937), for instance, were zoosporangia and not female gametangia. We must consider therefore, that it is only assumed, not proved, that thin-walled zoosporangia, as well as resistant sporangia, are borne in association with gametangia.

Until further evidence on this point is brought forward, rather than considering all plants which bear resistant sporangia as well as sex organs as gametophytic-sporophytic mixtures, it is, in the writer's opinion, simpler and more logical to adhere to Kniep's original concept of the two generations, gametophyte and sporophyte, remembering that the gametophytic hyphae as well as the sporophytic, often bear resistant sporangia.

Another question, which must be considered in this connection, is: what type of plants develop from R.S. zoospores discharged from resistant sporangia borne on sexual plants? Hatch (1935, p. 625) said that sexual myce-

lia could be derived from isolations of zoospores from resting sporangia formed on sexual mycelia, but he gave no experimental evidence in support of this statement. One would expect that R.S. zoospores derived from sexual plants, just as those derived from asexual plants, would usually develop into sexual individuals, but, in order to prove this point conclusively it will be necessary to make certain that each and every resistant sporangium from which the R.S. zoospores are derived was borne on a hypha which had also formed male gametangia. For the presence of pigmented male cells is, as we have seen above, the only valid criterion for distinguishing sexual plants. The writer has not yet isolated a sufficient number of resistant sporangia, of unquestionable gametophytic origin, to settle this question.

TABLE 2. Methods of Reproduction in *Euallomyces*. Bold face type shows the regularly recurrent methods; ordinary type shows the deviations from the usual life cycle; deviations which are questioned (?) were reported by Sörgel (1937) but have never been found by the writer.

REPRODUCTIVE "ORGANS"	SWARMERS	METHOD	PLANTS FORMED
Sporophytic			
1. **Zoosporangia**	→ **Zoospores**	→ **Without Fusion**	**Asexual**
		Without Fusion	? Sexual
2. **Resistant Sporangia**	→ **R.S. zoospores**	→ **Without Fusion**	**Sexual**
		Without Fusion	Asexual
		By Fusion With Each Other	? Asexual
Gametophytic			
1. **Male Gametangia**	→ **Male Gametes**	→ **By Fusion With Females**	**Asexual**
2. **Female Gametangia**	→ **Female Gametes**	→ **By Fusion With Males**	**Asexual**
		By Parthenogenesis	Asexual
		By Parthenogenesis	Sexual
3. Resistant Sporangia	→ R.S. zoospores	→ (Not Definitely Known)	

The various ways in which the four different types of uninucleate reproductive cells or swarmers may behave are summarized in the accompanying table which should help to clarify the somewhat complicated reproductive procedure which occurs in *Euallomyces*. It must be stressed again here that zoospores, R.S. zoospores and female gametes, are all nearly identical in size, structure, color (all lack pigment), and motility. They can be distinguished only by their place of origin or perhaps, to some extent, by their behavior, i.e., whether they react to and fuse with male gametes, how rapidly they germinate and grow, and what sort of plants they develop into. We know nothing at present concerning the exact ways in which the three sorts of swarmers differ from each other physiologically. As indicated above (p. 99) zoospores are probably diploid; R.S. zoospores as well

as female gametes on the other hand, are probably usually haploid. R.S. zoospores, however (from a few preliminary experiments which the writer has tried) will not react to or fuse with male gametes of the same strain. Hence, in spite of the close structural similarity there seems to be a marked physiologic difference between R.S. zoospores and female gametes. Further work along such lines should lead to a better understanding of these differences between the various types of swarmers.

Finally, it should be noted that nothing definite is known concerning the adjustments in chromosome number which must necessarily occur in conjunction with the various departures from the regular life cycle in *Euallomyces* which have just been reviewed. This problem will be considered in detail in connection with the genetical work on *Allomyces* which the writer hopes to publish in the near future.

Subgenus B. *Brachyallomyces*

When Kniep (1929) discovered the sexual stage of *A. javanicus* and showed that it arose directly from R.S. zoospores, it seemed probable that sexuality had been overlooked in previous investigations. This view was further strengthened by Weston's verification of sexual reproduction in his Philippine Island form, by Hatch's report of sexuality in *A. arbusculus* (1933), and particularly by the writer's discovery of sexuality in 43 of the 45 non-cyst-forming isolates in his collection. However, the remaining two isolates (Mexico 16, and India B2) *have, over a period of more than two years, failed to produce any sexual plants whatsoever.* Resistant sporangia of both these isolates have been germinated by the writer repeatedly, under widely different conditions, and after varying periods of "maturation" or drying. The methods used to obtain the sexual stage of some of the particularly obdurate forms of *Euallomyces*, have been tried time and time again with Mexico 16 and India B2 but entirely without success. The plants which developed from R.S. zoospores of these isolates, either in water or on agar, were *always* asexual; they bore zoosporangia and resistant sporangia but never any pigmented gametangia. Thinking that they might be isogamous forms in which both gametes were unpigmented and alike in size, careful observations were made to discover whether spores emerging from zoosporangia or resistant sporangia of these isolates might fuse with each other. Such fusions were never observed however; the swarmers always behaved as true spores, not gametes, and consistently germinated directly, after a swarm period, without any indications of sexual activity. The only plants produced by India B2 and Mexico 16 correspond exactly therefore with the asexual generation of *Euallomyces.* The complete cycle in these two isolates seems therefore to be a shortened one in which the sexual stage is entirely omitted and R.S. zoospores germinate directly into plants like their immediate parent. There is apparently no alternation of generations.

Now, as we have just seen certain isolates of *Euallomyces* give rise to sexual plants only very rarely or under special conditions; in these forms R.S. zoospores *usually* form asexual plants directly and, as in *Brachyallomyces*, there is a short-circuiting, so to speak, of the sexual stage. The sexual generation would certainly have been missed in China 2G, Texas 1, etc. had they not been studied intensively over a long period of time. We are, then, faced with the alternatives: (1) Will it always be possible, after numerous careful tests under a wide variety of conditions, to obtain sexual plants in all non-cyst-forming isolates? Or (2) Do some truly imperfect forms exist in which the R.S. zoospores *always* develop directly into asexual plants? In the writer's opinon a decision between these alternatives must, for the present at any rate, be almost arbitrary. But, mainly for convenience in future taxonomic studies of *Allomyces*, it seems best to adopt the second point of view tentatively and create a new species, *A. anomalus*, representing the subgenus *Brachyallomyces*. It should be emphasized that new isolates can only be placed in this species after thorough search has failed to reveal either cyst-formation or a gametophyte generation. If sexuality is subsequently discovered in these strains they will have to be placed in the appropriate species of *Euallomyces*. Wolf (1939) listed Texas 1 (from Stowell, Texas), China 2G (from Hupeh, China) and his own Mexican isolate 26 under *A. anomalus*, for at that time the sexual stage of these isolates had not been obtained. Since then the writer has found gametophytes with hypogynous male gametangia in all three strains, so it has been necessary to transfer them to *A. arbusculus* in *Euallomyces*. The writer has also found that, although Mexico 16 has failed to produce a sexual stage, Mexico 17, listed by Wolf (1939) as sp. indet., is *A. javanicus*. If, after several more years of study therefore, it is found that all non-cyst-forming isolates eventually do always reveal a sexual stage, then *Brachyallomyces* and *A. anomalus* may become invalid. However, Weston (unpublished notes) germinated resistant sporangia of his isolate from Alabama many times during the twelve years that he kept this strain in culture and never found indications of sexuality such as he discovered in his isolate from the Philippine Islands, and the writer's decision to place those forms of *Allomyces* with a short life cycle in a separate species, *A. anomalus*, is also strengthened by Miss Blackwell's report (1939 and 1940) of a similar brachyallomyces-type of cycle in the closely related genus *Blastocladia* and by the apparent absence of a sexual stage in *Blastocladiella simplex* Matthews (1937).

An interesting point arises as to the correct names of isolates of *Allomyces* which were studied before 1929 when Kniep reported sexuality in *A. javanicus*. Up to that time, with the exception of *A. moniliformis*, all the forms which had been investigated were included under *A. arbusculus*. As far as the writer can discover, no viable material remains from these

early isolations of *Allomyces* (starred in the list p. 81), and therefore it will never be possible to determine their complete life-cycles and classify them in accordance with our present knowledge: they must continue to remain in *A. arbusculus*. That some of them may in fact not have been *A. arbusculus* as we now define it (having a long life cycle and with hypogynous male gametangia) was brought out by a study of India B1, B2, B3, and B4. These strains were isolated from soil collected from the type locality at Pusa in India where Butler (1911) first found the type species of the genus, *Allomyces arbusculus*. India B2, as we have seen, has not produced any sexual stage and must be included in *A. anomalus*. Perhaps Butler's original *A. arbusculus* also lacked a gametophyte generation. Interestingly enough the other three of the writer's Indian isolates from Pusa, however, produce sexual plants on which the male cells in the primary pairs are borne terminally, i.e., male gametangia are epigynous. Hence, these isolates obviously belong in *A. javanicus*, and the interesting possibility appears that the original *A. arbusculus* of Butler may have had a sexual stage with the epigynous arrangement of gametangia which is the characteristic of *A. javanicus*. Obviously we can draw no definite conclusions from the evidence at hand. Since Hatch (1933) ascribed the hypogynous arrangement of gametangia to *A. arbusculus* we may as well retain the type species in that sense. It is interesting to note that *A. arbusculus*, the type species as we now define it, is the most wide-spread and frequently occurring species in the genus (see Fig. 16), and this is just as it should be.

Before concluding, it should be emphasized here again that we have, at present, no exact knowledge about the behavior of the nuclei in forms of *Brachyallomyces*. If the sporangium-bearing plants are diploid, then presumably in these short cycle forms, meiotic divisions fail to occur in the resistant sporangia and the diploid R.S. zoospores produce diploid asexual plants directly. Sörgel (1937) offered this suggestion to explain the short cycle deviations in *Euallomyces* described above. But the fact is that we do not know exactly how the nuclei behave either in *Brachyallomyces* or the short-cycle departures in *Euallomyces*.

Subgenus C. *Cystogenes*

Euallomyces and *Brachyallomyces*, as we have seen, are closely related since we can obtain the short brachyallomyces-type merely by removing the whole sexual stage from the euallomyces-cycle. As the writer first pointed out in 1938 and has since corroborated repeatedly, the third subgenus, *Cystogenes*, is markedly different however, and must be sharply separated from the other two on the basis of the unique structure and behavior of the R.S. zoospores.

Plants of *Cystogenes* (K, Fig. 7), like the asexual individuals of *Euallomyces* and all plants of *Brachyallomyces*, bear the two characteristic sorts

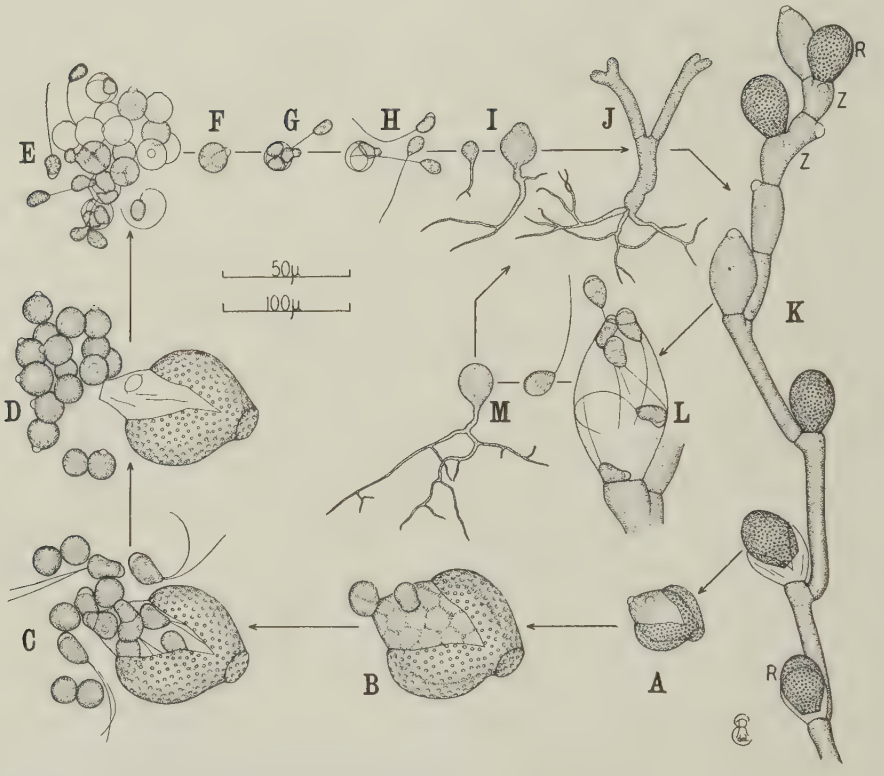


FIG. 7. The life cycle of *Cystogenes*, illustrated here with *A. cystogenus:* A, germinating resistant sporangium with outer wall split and two papillae of discharge formed on the inner membrane; B, the same (enlarged) just starting to release R.S. zoospores; C, primary R.S. zoospores emerging from the germinated resistant sporangium, some still motile, others already encysted and lacking flagella; D, cysts, clustered at the mouth of the empty resistant sporangium and each with a single papilla; E, secondary R.S. zoospores emerging from cysts; F–H, stages in the emergence of a quartet of secondary R.S. zoospores from a cyst; I, germinating secondary R.S. zoospore; J, young plant; K, hypha of a mature plant bearing thin-walled zoosporangia (Z, Z) and heavy-walled resistant sporangia (R, R); L, zoospores emerging from a zoosporangium; M, germinating zoospore. Upper scale for B–I, L and M, approximately × 335 as here reproduced; lower scale for A, J and K, approximately × 165 as here reproduced.

of sporangia, zoosporangia (Z, Z) and resistant sporangia (R, R). The ordinary zoospores are exactly similar in structure and behavior to those of all other members of the genus; they reproduce the parent plants directly and asexually (L and M). Although germination of the resistant sporangia (A and B, Fig. 7) in *Cystogenes* takes place just as in other forms, the spores produced (C) are very different: (1) a majority of them are characteristically biflagellate; (2) instead of swimming off for a more or less extended swarm period, they are motile for only a very short while and usually settle down, lose their flagella and encyst (C and D) in clusters near the parent sporangia within a few minutes after emergence; (3) they never germinate

by a tube as in the first two subgenera; instead, in the course of about 1–2 hours each cyst develops a small but typical discharge papilla, and a few cleavage planes become evident within; (4) very soon thereafter smaller, secondary, *monoflagellate* R.S. zoospores emerge from each cyst (E-H), usually in groups of four and, after a more or less extended swarm period, finally settle down, germinate in the usual way by a germ tube (I and J) and produce plants like the immediate parent. The process of encystment, which has just been outlined, is an absolutely regular and fundamental part of the life-cycle in these forms. There is no large and obvious sexual generation bearing anisogametes as in *Euallomyces* and the encystment of the primary R.S. zoospores clearly distinguishes *Cystogenes* from either *Brachyallomyces* or *Euallomyces*.

It is not yet apparent just how the life cycle in *Cystogenes* is to be interpreted. The monoflagellate secondary R.S. zoospores which emerge from cysts display no sexual activity: they do not fuse with each other but, on the contrary, develop directly, asexually, into new plants. The writer (1938) has pointed out the close similarity between the biflagellate primary R.S. zoospores of *Cystogenes* and the biflagellate planozygotes of *Euallomyces* and has suggested that sexual fusions may take place in *Cystogenes* during the germination of the resistant sporangia, i.e., primary R.S. zoospores may be zygotes. The formation of secondary spores in groups of four indicates furthermore that meiotic divisions may occur when the secondary spores are formed within the cysts. If these suggestions regarding the nuclear behavior prove to be correct it will be clear that the one obvious generation of plants in *Cystogenes* is haploid. A decision on this question must await a cytological study of the *Cystogenes* cycle.

It will be evident, from the foregoing discussion that one must observe the complete life-history of any new isolate of *Allomyces* before one can place that isolate definitely in its correct subgenus. The writer (1938) has shown, however, that certain characters may be of diagnostic value in tentatively classifying isolates whose life-cycles have not yet been determined. Thus, the resistant sporangia of all of the cyst producing forms which are known at present have more conspicuous and widely spaced pits than do those of any species in *Euallomyces* or *Brachyallomyces* (cf. Figs. 12–15). (A more detailed discussion of the pitting of resistant sporangia will be found in the section on specific characters.) Furthermore, members of the subgenus *Cystogenes* shed their mature resistant sporangia in great numbers through splits in the outer, encasing sheath (cf. the two lower resistant sporangia, K, Fig. 7). Resistant sporangia which have been shed in this way often form a conspicuous, brown halo on the bottom of the culture dish directly beneath plants which have remained undisturbed for several weeks. In *Euallomyces* and *Brachyallomyces* resistant sporangia are sometimes also released in this manner, but ordinarily a large majority of

them remain within their sheaths, attached to the parent hyphae. Aside from these small differences, as far as the writer has been able to discover, plants of *Brachyallomyces* and *Cystogenes* are so closely similar to sporophytes of *Euallomyces* that it is not possible to distinguish members of the three subgenera definitely in any way other than by determining their life cycles.

2. Criteria for Establishing Species and Varieties

With the basic differences between the three subgenera in mind we can turn now to a detailed consideration of the characters which have been used to distingusih the species and varieties of *Allomyces*. It will be well to emphasize at the start the writer's conception of specific and varietal differences within this genus. If a taxonomic scheme is to be useful and readily workable, species must, obviously, be defined in terms which are sufficiently clear-cut to enable other workers to give specific names to the isolates which they obtain. The writer does not believe that characters which intergrade completely in natural populations can be used satisfactorily to distinguish species. Such intergrading characters have, therefore, so far as possible, been reserved for making the less marked varietal distinctions. In the following discussion the more basic morphological characters will be taken up first under each sub-heading.

GAMETANGIA

Arrangement.—It was clear as early as 1930 from Kniep's observations that two distinct types of *Euallomyces* occur, differing from each other fundamentally in the arrangment of the terminal pairs of primary gametangia borne on the gametophytes. In Kniep's *A. javanicus* the male cells were regularly terminal in these primary pairs and the females were usually subterminal, i.e., the males were *epigynous*. In the sexual stage of another isolate which Kniep (1930) obtained from Bali the arrangement was just reversed: the female cells were characteristically terminal and the males were subterminal or *hypogynous*. Weston's form from the Philippine Islands on reexamination in 1930 proved to be in the hypogynous group and is, as we now know, closely similar to Kniep's Bali strain. A specific name was not given to these plants with hypogynous male cells, however, until 1933 when Hatch obtained the sexual phase of an isolate from North Carolina (N. C. 2) which, on the basis of its asexual stage had been placed in *A. arbusculus*. Hatch (1933) found that male gametangia were hypogynous in his isolate and described this type of gametophyte as the sexual stage of *A. arbusculus*. As we have seen (p. 108), there is some indirect evidence that Butler's original *A. arbusculus* may either have lacked a sexual stage or, like *A. javanicus*, have had a gametophyte with epigynous gametangia. Since there is no possibility of clearing up this question, however, we can simply and satisfactorily leave the matter as it now stands: *A.*

javanicus has epigynous male cells, *A. arbusculus* has hypogynous male cells. In this way the two species are sharply defined.

Further work has shown however, that the situation is not quite as simple as this. Kniep himself noticed that in *A. javanicus* male gametangia were occasionally hypogynous in the terminal pairs and he figured (1929, p. 206) a pair of gametangia with this type of reversed arrangement. The writer's own observations, summarized in Table 3, are in agreement with

TABLE 3. Showing the Arrangement and Frequency of Male and Female Gametangia in *A. javanicus*. 1—the frequency of occurrence of primary pairs with two males (MM), an epigynous male and a female (MF), a hypogynous male and a female (FM), and two females (FF). 2—the ratio between the total number of males (M) and the total number of females (F), including all cells single or paired. The results in each case are based on 500 counts of gametangia taken from agar cultures 2 to 4 weeks old.

Isolates	1—Primary pairs, in %				2—Total, single or paired, in %	
	MM	MF	FM	FF	M	F
Var. *javanicus*						
Java 1	7.0	90.2	0.8	2.0	57	43
	2.2	96.0	0	1.8	46	54
	2.8	93.0	3.0	1.2	58	42
Fiji D_3	2.6	97.0	0.2	0.2	48	52
Mexico 17	5.0	94.4	0.4	0.2	54	46
India B_3	16.0	83.0	0.4	0.6	72	28
Tanganyika 3A	20.0	72.6	4.2	3.2	38	62
Var. *perandrus*						
Fiji D_2	93.6	6.0	0.4	0	96	4
Var. *macrogynus*						
Burma 1Da	1.6	97.6	0	0.8	57	43
Burma 3	2.8	97.2	0	0	53	47
Texas 2	3.2	96.8	0	0	55	45
India B4	7.8	92.0	0	0.2	57	43

those of Kniep and show moreover that the aberrant arrangement of gametangia occurs with greater or lesser frequency in different isolates: in *A. javanicus* var. *macrogynus* the hypogynous arrangement has never been found whereas in certain isolates of *A. javanicus* var. *javanicus* it appears quite often. In *A. arbusculus* on the contrary, as far as the writer can discover, the formation of epigynous males is very rare in all isolates, and hence this character can not even be used to distinguish varieties of the species.

Table 3 also shows another character which differs considerably in different isolates of *A. javanicus;* that is, the relative frequency of the occurrence of two male or two female gametangia, rather than a male and a female together, in the primary pairs. Thus in Fiji D_2 there are far more paired males

than in Java 1 or Fiji D_3; other strains are intermediate and a fairly complete series can be arranged. Since this is a quantitative difference showing regular gradation in a natural population it does not seem possible to draw any specific distinctions on such grounds. There is such a marked preponderance of male cells in Fiji D_2 however (96 per cent males, 4 per cent females), that this isolate has been given the varietal name *perandrus*. Agar cultures of *perandrus* are a particularly brilliant orange because a great majority of the reproductive cells are pigmented male gametangia.

In conclusion then, it can be said that the arrangement of the sexual cells of *A. arbusculus* is so closely similar in all isolates that no varietal distinctions can be made, whereas in *A. javanicus* on the contrary the number and arrangement (as well as size ahd shape, see below) of gametangia appear to differ markedly enough to divide the species into three varieties:

1. var. *javanicus* with approximately equal numbers of male and female cells, and primary males sometimes hypogynous;
2. var. *perandrus* with a great preponderance of male cells;
3. var. *macrogynus* with approximately equal numbers of male and female cells, but primary males never hypogynous.

The writer has effected interspecific crosses between *A. arbusculus* and *A. javanicus* (cf. p. 98) and obtained a large number of hybrid strains some of which are intermediate in many of their characters, gametangium-arrangement, etc., between the parental species. The bearing of this work on problems of taxonomy in *Allomyces* is considered in the general section on intergradation (p. 136).

Size and Shape.—These characters are very variable indeed. Environmental conditions, and particularly nutrient-supply, strongly affect the absolute size of gametangia as well as sporangia and hyphae. Abnormally small gametangia are sometimes formed by germlings in weak nutrient solutions (see Fig. 8 f and g). Under normal conditions there are probably fairly constant but slight differences between the mean size of gametangia of some of the isolates, but these differences do not fall in clear-cut groups nor are they sufficiently large to make even varietal distinctions. A detailed study of the size of resistant sporangia is discussed below, and the general conclusions reached apply equally to a consideration of the other reproductive organs.

In general it may be said that the primary female gametangia of *A. arbusculus* tend to be subspherical, probably owing partly to their terminal position, while those of *A. javanicus* are usually more nearly cylindrical or barrel-shaped. This difference is not by any means a constant one however.

In Kniep's form (Java 1) and the writer's other isolates of *A. javanicus* var. *javanicus* female gametangia in the primary pairs are usually not more than slightly longer than the accompanying males (cf. Fig. 8, a–e).

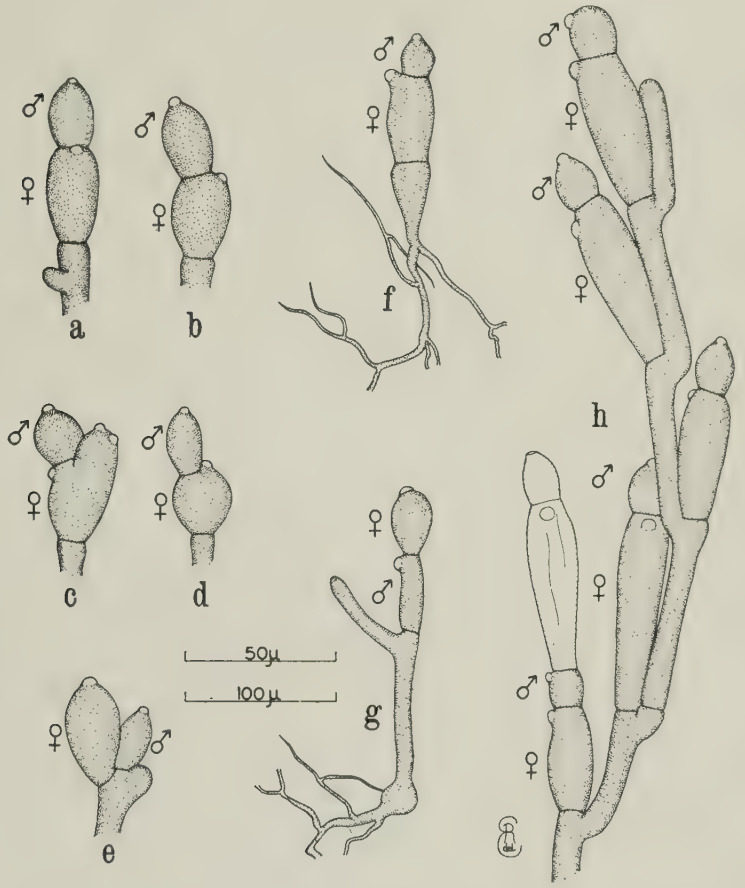


FIG. 8. a–e, primary pairs of gametangia of *A. javanicus* var. *javanicus* showing characteristic shapes and arrangements. (Gametangia of this sort also occur in older cultures of var. *macrogynus*.) f, abnormally small, mature, gametophytic plant of *A. javanicus* var. *macrogynus* formed in very weak nutrient solution; g, the same of *A. arbusculus*. h, mature gametophytic hypha of *A. javanicus* var. *macrogynus* bearing characteristic pairs of gametangia; one primary pair is empty and a secondary pair has been formed below. Upper scale for f and g, approximately × 400 as here reproduced; lower scale for a–e and h, approximately × 200 as here reproduced.

The gametangia are, moreover, often irregular in shape and arrangement and are frequently formed in long chains. In *A. javanicus* var. *macrogynus*, on the other hand, primary male and female gametangia are very regularly paired, and in young, pure cultures, particularly in water, the female cells are strikingly elongate, nearly cylindrical, and often 3 to 4 or even 5 times as long as the small epigynous males (cf. Fig. 8, h and Fig. 11). The writer believed at one time that the regularity in arrangement of gametangia, coupled with the large size of the female cells might warrant placing the isolates Burma 3, Burma 1Da, India B4, and Texas 2 in a separate species,

A. macrogynus. (This specific name was, in fact, used by Emerson and Fox, 1940.) However, both types, var. *javanicus* and var. *macrogynus* approximate each other in older cultures and the differences which have been observed thus far do not seem to be sufficiently sharp or consistent to allow of more than varietal distinction at present.

Pigmentation.—The quantity of pigment formed in the male cells of any isolate of *Euallomyces* varies considerably and is partly dependent upon the environmental conditions. Newly formed male cells borne on young, vigorous plants growing in pure water culture frequently discharge gametes before becoming even faintly colored. Pigment-formation is apparently a cumulative process, and hence brightly colored gametangia occur particularly under conditions in which the emergence of the gametes is temporarily prevented, as, for instance, in old liquid cultures where the concentration of organic matter is high or in agar cultures where the lack of moisture as well as the concentration of nutrient prohibit gamete emergence. Here we find the most brilliantly colored male cells, probably containing more pigment than is ever accumulated under natural conditions. This variation of color with age is found in all forms of *Euallomyces*. Nevertheless, certain slight but characteristic color differences between individual isolates are regularly found and show up particularly well in cultures of sexual plants, three to four weeks old, grown on Y p.S s. agar, where maximum pigment accumulation occurs. A list of typical examples is shown below, in each species arranged in a sequence passing from the more brightly to the less brightly pigmented forms. While certain isolates of *A. arbusculus*, such as Ceylon 1, Mexico 37, etc., are closely similar in color to some forms of *A. javanicus*, the pigment in the former species is usually markedly less brilliant than in the latter. Beyond this general statement no further distinctions can be made, since there is such close and delicate intergradation in color as comparison with Ridgway's Color Standards will show.

A. javanicus	*A. arbusculus*
Fiji D_2—Orange. (The particularly intense color of this isolate results partly from the preponderance of male cells.)	Ceylon 1—Capucine Yellow.
Fiji D_3 and Java 1—Capucine Yellow.	Mexico 37—Ochraceous Orange.
Burma 3 and Burma 1 Da—Zinc Orange.	Burma 1A and Philippine Isls. 1—Light Ochraceous-Salmon.
	Louisiana 1—Pale Yellow-Orange.
	Fiji B_1—Capucine Buff.

(Colors based upon Ridgeway's *Color Standards and Nomenclature*, Washington, 1912.)

Emerson and Fox (1940) recently extracted and analysed the pigment from a number of isolates of *A. arbusculus* and *A. javanicus* and found that in both species the color is due primarily to the presence of γ-carotene. Traces of the β-isomer were also found in Fiji D_2.

Resistant Sporangia

The occurrence and morphology of the resistant sporangia appear to be

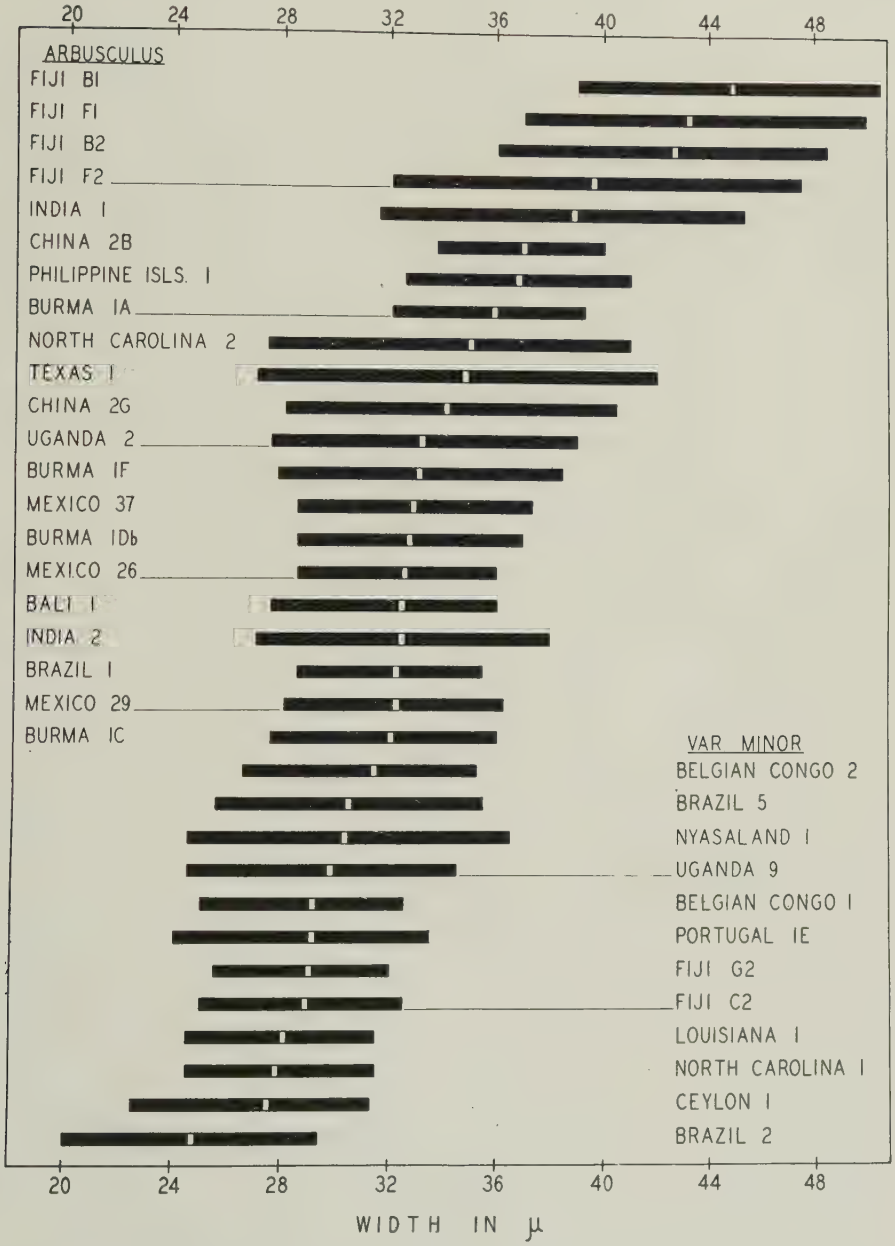


FIG. 9. Intergradation in the size, i.e. width here, of resistant sporangia of *A. arbusculus*. Each bar shows the limits which include the median 75% of a random sample of resistant sporangia of each isolate. The white blocks indicate the value of the arithmetical mean. For further details refer to the section on *A. arbusculus* in Table 4.

TABLE 4. Size of Resistant Sporangia in *Allomyces*.

N is the number of sporangia measured; M is the arithmetical mean with the probable error; σ is the standard deviation.

ISOLATE	N	WIDTH			LENGTH			Length: Width
		Range	M	σ	Range	M	σ	
var. *arbusculus*								
Fiji B1	100	30–57	44.9±.3	4.66	48–81	64.8±.4	6.60	1.44
Fiji F1	100	28–55	43.2±.4	5.84	36–69	55.8±.4	6.40	1.29
Fiji B2	100	30–59	42.7±.3	5.00	42–73	59.5±.4	5.98	1.39
Fiji F2	150	26–51	39.6±.3	5.96	34–67	51.1±.4	7.38	1.29
India 1*	28	24–49	38.9±.8	6.14	32–59	49.1±.7	5.30	1.26
China 2B	200	24–45	37.0±.1	2.80	32–67	56.7±.2	4.16	1.53
Philippine Is. 1	200	26–47	36.8±.2	3.64	38–63	51.0±.2	4.10	1.39
Burma 1A	200	28–47	35.9±.2	3.16	32–67	50.2±.3	5.58	1.40
North Carolina 2	200	16–45	35.0±.3	5.60	28–67	51.1±.3	6.92	1.46
Texas 1	100	24–47	34.8±.4	5.54	30–69	48.7±.5	7.86	1.40
China 2G	100	20–47	34.1±.4	5.28	36–73	47.8±.4	6.30	1.40
Uganda 2	100	22–45	33.2±.3	4.62	32–69	46.0±.5	6.90	1.39
Burma 1F	150	22–45	33.1±.2	4.32	34–55	44.8±.2	4.38	1.35
Mexico 37	100	22–45	32.8±.3	4.20	32–57	44.1±.3	4.48	1.34
Burma 1Db	200	20–43	32.7±.2	3.70	28–55	42.7±.2	4.82	1.31
Mexico 26	100	22–39	32.5±.2	3.14	32–51	43.3±.2	3.64	1.33
Bali 1	200	18–41	32.4±.2	4.00	30–65	47.5±.3	5.94	1.47
India 2	150	22–45	32.4±.2	4.46	28–57	41.6±.3	5.20	1.28
Brazil 1	100	26–39	32.2±.2	2.84	38–53	43.4±.2	2.84	1.35
Mexico 29	100	20–41	32.2±.2	3.52	32–53	42.2±.2	3.56	1.31
Burma 1C	100	24–47	32.0±.3	3.82	32–59	44.9±.3	4.88	1.40
var. *minor*								
Belgian Congo 2	150	20–39	31.4±.2	3.50	24–55	39.7±.2	4.60	1.26
Brazil 5	100	22–41	30.4±.3	3.96	26–61	38.3±.4	5.66	1.26
Nyasaland 1	150	18–41	30.3±.3	4.90	30–63	46.8±.4	6.34	1.54
Uganda 9	100	18–39	29.8±.3	4.00	28–53	40.4±.3	4.46	1.36
Belgian Congo 1	100	20–35	29.1±.2	2.80	30–55	41.6±.3	3.80	1.43
Portugal 1E	100	22–39	29.1±.2	3.60	32–51	40.2±.2	3.58	1.38
Fiji G2	100	18–39	29.0±.2	3.14	24–51	39.7±.3	4.44	1.37
Fiji C2	100	20–37	28.9±.2	3.10	26–51	39.1±.4	5.36	1.35
Louisiana 1	200	20–37	28.1±.1	2.74	24–47	36.8±.2	3.74	1.31
North Carolina 1	200	16–37	27.8±.2	3.28	20–47	35.4±.2	4.66	1.27
Ceylon 1	200	16–37	27.5±.2	3.68	22–49	35.4±.2	4.76	1.29
Brazil 2	100	18–35	24.7±.2	3.54	24–43	34.0±.2	3.74	1.38
A. javanicus var. *macrogynus*								
Texas 2	100	28–53	41.9±.3	4.12	44–73	61.4±.4	5.80	1.46
India B4	100	26–53	41.4±.4	5.14	36–73	57.9±.4	6.66	1.40
Burma 1Da	150	28–53	40.6±.2	4.52	42–85	64.1±.4	7.62	1.58
Burma 3	200	26–51	39.3±.2	5.04	32–69	54.6±.4	7.56	1.39

* From a slide of fixed type-material prepared by E. J. Butler and sent to W. H. Weston.

TABLE 4. (*Continued*)

ISOLATE	N	WIDTH Range	WIDTH M	WIDTH σ	LENGTH Range	LENGTH M	LENGTH σ	Length: Width
var. *perandrus*								
Fiji D2	150	26–53	37.7±.3	6.14	38–73	55.2±.4	7.62	1.46
var. *javanicus*								
Mexico 17	150	28–53	41.2±.3	5.38	38–67	53.7±.3	5.68	1.30
Fiji D3	150	24–51	36.8±.3	5.64	38–67	51.8±.3	5.62	1.41
Java 1	200	22–47	36.5±.2	4.40	30–57	44.2±.2	4.60	1.21
India B3	100	24–49	35.2±.3	4.66	32–57	45.4±.3	4.52	1.29
Tanganyika 3A	200	20–49	35.1±.3	5.48	30–73	46.8±.4	7.50	1.33
A. anomalus								
Mexico 16	160	24–51	41.0±.3	5.68	34–71	54.3±.3	6.24	1.32
India B2	150	26–55	39.5±.3	5.58	40–87	64.3±.5	8.30	1.63
A. cystogenus								
var. *cystogenus*								
Burma 1B	200	24–49	37.7±.2	3.28	36–73	50.9±.3	6.38	1.35
China 2H	100	28–43	35.6±.2	2.94	40–77	54.3±.4	6.28	1.52
Venezuela 1	150	24–43	33.2±.2	3.40	34–95	55.1±.6	10.20	1.66
var. *elongatus*								
China 2J	200	26–41	35.1±.1	2.56	54–85	71.4±.3	5.90	2.03
A. moniliformis								
Mexico 46	100	26–39	34.4±.2	2.48	44–87	62.5±.4	6.64	1.82
North Carolina 3	156	22–37	30.6±.2	3.04	38–79	53.1±.4	6.54	1.74

characters which are relatively constant in a single isolate or group of isolates. For this reason the writer has made a particularly extensive comparative study of the resistant sporangia to discover to what extent their characters might be of use in classifying the various forms.

Size and Shape.—In standard hemp seed water-cultures (cf. Methods, p. 85) resistant sporangia of any particular isolate vary in size within certain quite well defined limits as shown in Table 4. The measurements (Table 5) of resistant sporangia obtained from cultures of the Philippine Islands form of *A. arbusculus* grown at different temperatures and in different amounts of water or on agar indicates that the mean size of resistant sporangia of a single isolate may vary considerably (as much as 5 or 6μ in mean width) in a series of cultures grown under different conditions, yet under any set of fairly standard and comparable conditions each isolate is characterized by a quite well defined mean size of resistant sporangia. The size of these structures might therefore be a character of taxonomic value. When, however, the isolates are compared as shown in Fig. 9, the very interesting fact appears that they can be arranged in a complete series ranging from the largest (mean width 45μ) to the smallest (mean

TABLE 5. Variation in Size of Resistant Sporangia of *A. arbusculus*, Isolate Philippine Islands 1.*

CULTURE	N	WIDTH			LENGTH			Length: Width
		Range	M	σ	Range	M	σ	
Water								
Room Temp. —	200	26–47	36.8±.2	3.64	38–63	51.0±.2	4.10	1.39
25°C. Crys. dish	200	22–49	35.6±.2	5.14	35–61	49.5±.3	5.42	1.39
25°C. Qt. Jar	200	22–45	35.4±.2	4.54	32–65	48.6±.3	6.16	1.37
30°C. Crys. dish	200	26–47	37.0±.2	3.44	38–59	51.1±.2	4.16	1.38
30°C. Qt. jar	200	24–47	37.0±.2	3.58	30–65	51.3±.2	4.62	1.39
35°C. Crys. dish	200	22–49	32.4±.2	4.82	30–65	46.9±.3	6.08	1.45
35°C. Qt. jar	200	24–45	35.3±.2	4.22	34–65	50.0±.2	5.16	1.42
Agar								
Rm. temp., Ye.S.	50	32–47	37.7±.3	2.92	42–57	49.1±.3	3.32	1.30
Rm. temp. oatmeal	200	26–45	34.5±.2	3.62	34–59	47.8±.2	5.16	1.39

* I am very much obliged to Miss Susan Hedge for her help with this series of measurements.

width 25μ) but with a difference of never more than 4μ between adjacent strains. If one wishes to use size of resistant sporangia as a taxonomic character where is one to draw the line in such a series? Obviously from a study of only a small number of isolates one might easily conclude that two sharply defined groups exist, one for example with resistant sporangia having a mean width of 25–30μ, the other 40–45μ. According to the accepted concept of a species in the fungi such a difference might well be considered of specific value. Yet, where further study, as so frequently happens, reveals a complete series of intermediates the original specific distinctions are much less tenable if not quite worthless.

In the writer's opinion, attempts to use these closely intergrading characters, such as size of resistant sporangia in *Allomyces*, to define species are bound to lead to confusion. He believes, therefore, that the present problem will be simplified and clarified for the future if all isolates of *Euallomyces* which characteristically have hypogynous male gametangia are included in the species *A. arbusculus*. Since, however, this species is a large one, including a majority of all the isolates (32 of the 51 in the writer's collection), it has been of value to divide it arbitrarily into two varieties, *arbusculus* with large resistant sporangia (the type variety since it includes the type isolate, India 1) and *minor* with small ones, clearly understanding at the same time that one variety grades directly into the other.

A similar, although less complete series of isolates of *A. javanicus* is also shown in Table 4. It is apparent that the character resistant sporangium size can not, at least for any of the forms which we now know, be used to distinguish species definitely. All we can say is that no isolates of *A. javani-*

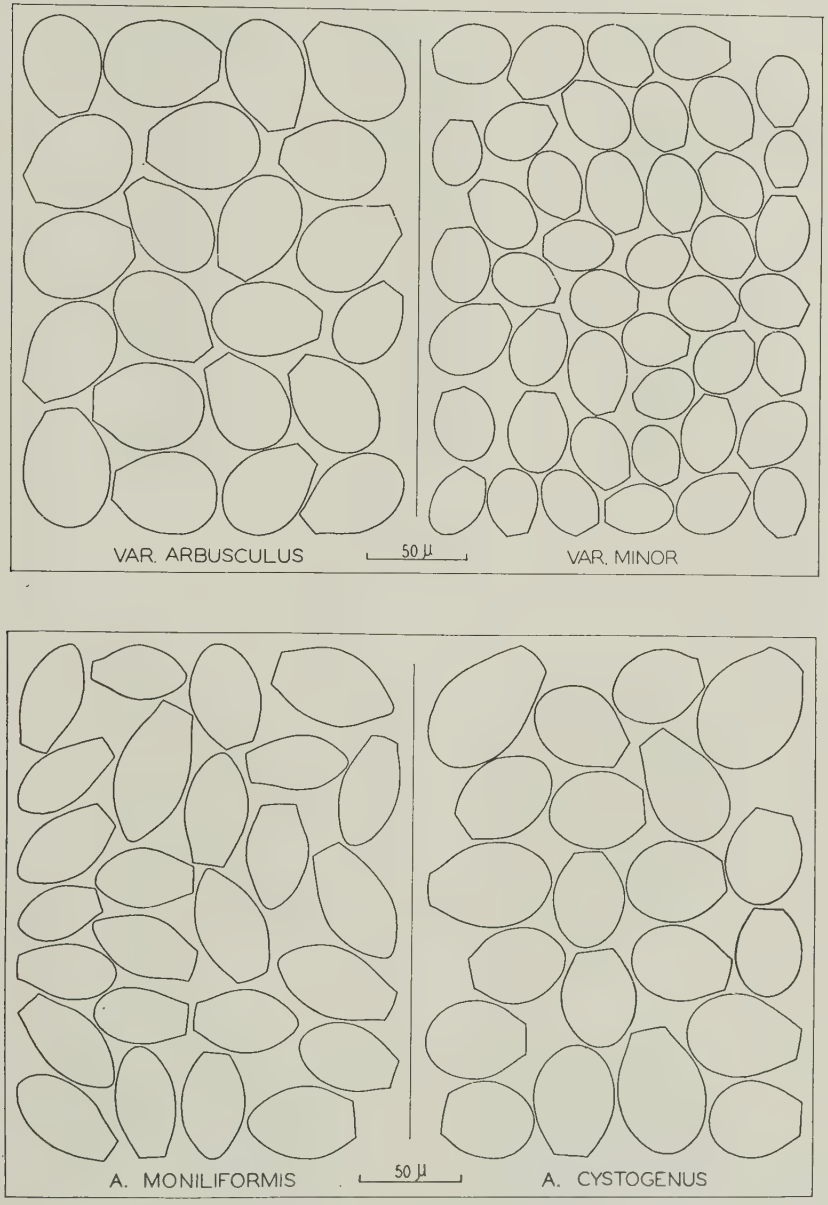


FIG. 10. Comparison of the size and shape of resistant sporangia: above, *A. arbusculus* var. *arbusculus* (Philippine Islands 1) and var. *minor* (Louisiana 1); below, *A. moniliformis* (North Carolina 3) and *A. cystogenus* var. *cystogenus* (Burma 1B). Camera lucida outlines made from a random sample of resistant sporangia of each isolate. Approximately × 250 as here reproduced.

FIGS. 11–15. *(See opposite page for explanation.)*

cus or the cyst-forming species are known at present which have such small resistant sporangia as do some of the smaller forms of *A. arbusculus* var. *minor*.

In general, the remarks which have just been made about the size of resistant sporangia apply also, with one major exception, to a consideration of the shape of these structures. As shown in Fig. 10, the writer has found that a majority (about 70% in any random sample) of the resistant sporangia of *A. moniliformis* have a bluntly pointed apex (like that of *Blastocladia rostrata* von Minden, 1916) in contrast to the broadly rounded apex of the closely related species *A. cystogenus* and all other known isolates of *Allomyces*. Otherwise all the isolates of *Allomyces* in the writer's present collection have more or less ovoid resistant sporangia with a flat sometimes slightly truncate base. In both *A. javanicus* and *A. arbusculus* forms occur which regularly have resistant sporangia tending either toward a more subspherical shape with a less truncate base (Java1, Burma 1Db) or a more elongate shape (Burma 1Da, China 2B, etc.), and in both species there are, as shown by the length/width ratios in the last column of Table 4, intermediate types. In *A. cystogenus* one isolate (China 2J) has resistant sporangia which are so markedly elongate (averaging twice as long as broad) as to warrant placing this form in a separate variety, *elongatus*.

Pitting in the walls of resistant sporangia is very similar in all closely related species discovered thus far, and is not in itself usually of any definite diagnostic value. The slight differences which do occur are shown in Figs. 12–15. All cyst producing forms have large rather widely spaced pits. The pits in *A. arbusculus* var. *minor*, particularly the smaller forms, are sometimes also quite large and widely spaced. In *A. javanicus* var. *macrogynus* on the other hand the pitting may be very fine and is sometimes almost indistinguishable except under the highest magnifications. There is a good deal of variation within any one isolate but the differences outlined above are useful in tentatively assigning a specific name to a new isolate whose complete life cycle has not yet been worked out.

Color differences, too, are not really distinctive, particularly as color varies with age and with different nutrients. Masses of resistant sporangia in old water cultures are a characteristic tawny brown (Ridgway's Buckthorn Brown or Ochraceous-Tawny); when maturing, they may be more

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FIG. 11. Photomicrographs of primary pairs of gametangia of *A. javanicus* var. *macrogynus* from a young water culture: a, mature and ready to discharge; b, male cell (terminal) nearly empty female discharging; c, both cells empty. All approximately × 275 as here reproduced; each division of the scale represents 10μ.

FIGS. 12–15. Photomicrographs of germinated and empty resistant sporangia showing the size and distribution of pits in the outer wall: 12, *A. arbusculus* var. *arbusculus* (Philippine Islands 1); 13, *A. arbusculus* var. *minor* (Louisiana 1); 14, *A. javanicus* var. *macrogynus* (Burma 3); 15, *A. cystogenus* var. *cystogenus* (Burma 1B). All approximately × 560 as here reproduced; each division of the scale represents 10μ.

nearly pink or brick-red; formed on Ye.S. agar in great numbers they color the cultures a deep chocolate brown (Ridgway's Mikado Brown) while those produced on oatmeal agar are sometimes very pale, grading to straw-color. Resistant sporangia of Java 1 are often more olivaceous (Ridgway's Buffy Brown) and differ in this respect from those of other isolates.

The properties of the brown pigment in the heavy, outer walls of the resistant sporangia indicate, as Emerson and Fox (1940) have shown, that it is probably a melanin-like substance.

Occurrence.—It was early recognized (Barrett, 1912) that zoosporangia are normally formed first on young hyphae, heavy-walled sporangia appearing somewhat later depending on environmental conditions. In standard water cultures all isolates except Java 1 start to produce resistant sporangia within a few days after inoculation, and in the course of three or four weeks become brown with great numbers of them. Plants of Java 1, on the other hand, remain macroscopically pale gray under similar conditions and can only be induced to form a comparable crop of resistant cells by removing most of the water from the cultures and (or) heating them to 30°–40°C. for some days. This peculiar scarcity of the resistant sporangia, combined with their olivaceous-brown color, distinguishes Java 1 from the other isolates of *A. javanicus* var. *javanicus*, to which it is closely similar in all other ways.

ZOOSPORANGIA

Size and Shape.—Thaxter (1896), von Minden (1916) and others have noted that the size and shape of zoosporangia vary greatly in species of *Blastocladia*. In *Allomyces*, too, these structures are quite variable. Their size, in all species, is closely dependent upon the amount of available nutrient: germlings in pure water may produce small sporangia, less than 10μ in diameter, and in extreme cases zygotes have been observed to function directly as minute sporangia after sending out a few rhizoids. Shape depends on a variety of factors; zoosporangia (and gametangia) formed in agar or rich liquid nutrient tend to be less elongate and sometimes nearly spherical, while those developed in pure water, on young vigorous hyphae, and discharged immediately are usually more nearly ellipsoid or cylindrical. Zoosporangia of species in the subgenus *Cystogenes* are, in general, more elongate than they are in other forms, but beyond this no valid distinctions can be made. Secondary zoosporangia in all of the species are frequently borne basipetally in chains of varying length, but this character is particularly marked in *A. moniliformis*.

Pigmentation.—As far as the writer has been able to discover, orange pigment never appears within the cytoplasm of *Brachyallomyces* or asexual plants of *Euallomyces*. Emerson and Fox (1940) were unable to detect any traces of carotenoids in extracts of the asexual stage of *Euallomyces* grown

on Yp. Ss. agar, although complete extraction was always indicated by the demonstration of sterols.

The localization of carotene in the male cells of *Euallomyces* is very striking and consistent. The writer was surprised, therefore, to discover that in isolates of *A. moniliformis*, a cyst producing species lacking an obvious sexual generation, orange pigment sometimes appeared in oil droplets of varying size, distributed irregularly here and there in the cytoplasm of hyphae as well as thin-walled sporangia and resistant sporangia. Believing that the pigment might indicate the presence of gametangia and gametes such as occur in *Euallomyces*, some of the zoosporangia which contained particularly large quantities of pigment and looked very similar to male gametangia of *Euallomyces*, were isolated singly in hanging drops of water. In every case the spores which emerged were large, 10–12μ in mean diameter, and behaved exactly as ordinary zoospores, i.e., they showed no indications of sexual fusion and, after a swarm period, rounded up and germinated in the usual way. Spores from isolated sporangia were plated out on nutrient agar and always produced plants of the characteristic *A. moniliformis* type. There can be no doubt, therefore, that although the spores were pigmented, they are not otherwise comparable in any way with male gametes of *Euallomyces*. The latter are much smaller and are unable to germinate into new plants unless they fuse with female gametes, necessarily from another gametangium.

A. moniliformis is distinguished, therefore, from all other species of *Allomyces* by this formation of pigment in the hyphae and sporangia. The color does not appear under all conditions however; it is usually lacking in water cultures until the plants are several weeks old, but it appears in large quantities in plants grown on oatmeal agar. In fact, in stock cultures of *A. moniliformis* (particularly Mexico 46) grown on oatmeal agar slants, so much pigment is accumulated that the brown color usually imparted by the resistant sporangia becomes completely masked and the cultures look very much like sexual growths of *Euallomyces*. Emerson and Fox (1940) found that the pigment in *A. moniliformis* is, like that in the male cells of *Euallomyces*, primarily γ-carotene. They were unable to detect any traces of orange pigment in the closely related species *A. cystogenus* which can therefore be separated from *A. moniliformis* on this pigmentary difference as well as on the difference in shape of resistant sporangia noted above.

SWARMERS

Even though zoospores and gametes, from any one isolate, vary only slightly in size, differences throughout the genus from one form to another, do not appear sufficiently striking to be of value in separating any of the closely related species or varieties known at present. This character may prove to be important in the future when new forms are found; the gametes

of *A. arbusculus* (females 8–11.5μ, males 5.5–8.5μ) are, on the average, more nearly isogamous than those of *A. javanicus* (females 9–12μ, males particularly active, 4–6μ), and truly isogamous forms of *Allomyces* may be discovered by later investigators.

In addition to occasional, semiamoeboid activity, all the types of swarmers, when free swimming may present shapes varying from long ellipsoid, to lacrymoid (blunt anteriorly or posteriorly) to quite spherical. These changes are probably related to surface tension phenomena, and no particular shape is constantly characteristic of any given isolate.

Taxonomic Outline

Limits of the order

The order Blastocladiales was first established by Petersen (1910), who included in it but one family, the Blastocladiaceae, represented at that time by a single genus, *Blastocladia*. *Allomyces* was discovered the following year by Butler (1911) and placed in the Blastocladiaceae by von Minden in 1916, while the genus *Blastocladiella* was described by Miss Matthews and added to the group in 1937. Stüben (1939) has recently described a new genus, *Sphaerocladia*. Although the character and method of germination of the resistant sporangia and the peculiar structure of the "food-bodies" in the swarmers of this fungus make it necessary to widen somewhat our concept of the Blastocladiales, *Sphaerocladia* is basically so similar to the other genera of the order that it should quite certainly be included here as Stüben has done. Strictly defined at present therefore the order is, in the writer's opinion, composed of but four genera.

The enigmatical genus *Gonapodya* Fischer (1892), although sometimes placed in the Blastocladiales (Kanouse 1927, Fitzpatrick 1930, Coker 1937), has not been included here. It apparently lacks the resistant sporangia so characteristic of all other members of this group and, for the present until more is known about its life cycle, might better be placed in the Monoblepharidaceae as Sparrow (1933) has done. *Mindeniella* Kanouse (1927) is also a problematical genus; it was not included in the Blastocladiales by Fitzpatrick (1930) and can not be considered a member of this order until it has been studied more thoroughly. Characteristic blastocladiaceous resistant sporangia were not found, nor were zoospores described, and Kanouse reported that the cell walls, in contrast with those of all other known Blastocladiaceae, turned blue when treated with chloriodide of zinc. Coker (1937) retained *Mindeniella* in the Blastocladiales, but it is certainly more closely related to *Araiospora* as Sparrow (1935a) has suggested.

As a general basis for the present analysis of the Blastocladiales the writer has used von Minden's (1916) critical and, up to that time, inclusive description of the order, with fundamental corrections and additions per-

taining particularly to the two new genera and the processes of reproduction which have since been discovered. For purposes of comparison with *Allomyces* brief outlines of the characters which distinguish the other three genera are also included here.

Blastocladiales Petersen 1910

Fresh water aquatics, saprophytic on submerged plant and animal substrata, sometimes found as soil inhabitants. Cell walls composed of chitinous rather than cellulose-like materials. *Thallus* nonseptate or segmented by pseudosepta; simple, or, when branched, usually divisible into a stouter basal portion or axis and more slender, secondary, fertile branches; basal portion firmly attached to the substratum by a finely ramified system of tapering rhizoids; branching irregular to purely dichotomous, sometimes nearly umbellate, often clearly sympodial in the reproductive part. *Reproductive organs:* (1) zoosporangia with a simple, thin, hyaline and colorless wall (the original hyphal membrane) and one to several conspicuous exit papillae; discharging zoospores which reproduce the plant asexually; (2) resistant sporangia similar to zoosporangia in mode of formation and usually in position but differing markedly in final structure; produced within the hyphal membrane, which may remain as an outer sheath; with a very characteristic double wall, the outer layer usually brown, thickened, and interspersed with pits (except in *Sphaerocladia*), the inner thin and membranous; when mature sometimes falling off as a whole still excased in the hyphal sheath or slipping out through a split in the latter; germinating by cracking of the outer, heavy wall and swelling of the content surrounded by the thin inner membrane on which are formed one to several discharge papillae; producing swarmers, R.S. zoospores, which, except in *Allomyces* subgen. *Cystogenes*, develop directly into new plants; (3) gametangia known in *Allomyces* subgen. *Euallomyces*, *Blastocladiella variabilis* and *Sphaerocladia;* closely similar to zoosporangia in mode of formation, in position, and in final structure; borne on sexual plants arising from R.S. zoospores; discharging planogametes which fuse in pairs to produce planozygotes. Zygotes germinating immediately without any period of "rest." *Swarmers*—i.e., zoospores, R.S. zoospores (except primary R.S. zoospores of *Allomyces* subgen. *Cystogenes*), and gametes—ellipsoid to subspherical; with a swarm period, monoplanetic, and sometimes semiamoeboid; not discharged in a true vesicle; with a single, posterior flagellum and a centrally located, subtriangular nuclear cap; nucleus imbedded in the latter and situated in the posterior end near the point of attachment of the flagellum; cytoplasm hyaline, usually containing obvious lipoid granules or oil droplets. All zoospores (except primary R.S. zoospores of *Allomyces* subgen. *Cystogenes*) and zygotes germinate directly by a germ tube.

BLASTOCLADIACEAE Petersen 1910

Characters of the order. (This is the sole family.)

I. *Blastocladia* Reinsch 1878

Characters of the family. Plants known only on vegetable substrata, usually in dense pustules; never more than a few mm. in height.

Thallus unicellular, without pseudosepta or constrictions; often consisting of a simple, and sometimes greatly swollen, main axis, which bears the sporangia directly; or, the main axis less strikingly developed, more nearly cylindric and branching above, the fruiting organs borne on ultimate branches, the tips of which are often swollen; branching irregular, subdichotomous or subumbellate; sometimes bearing fine, sterile threads of unknown function.

Zoosporangia very rarely catenulate (in pairs); borne terminally and then often arranged sympodially, but also sometimes closely crowded together on a simple axis or on shortened branches; in one species renewed by proliferation; usually elongate, clavate, fusiform, or cylindrical but sometimes globose; with a single apical exit papilla, the latter sometimes with a marked, cone-shaped plug-like projection extending inwards; often dropping off before or after zoospore-discharge, leaving circular scars.

Resistant sporangia as above in formation and position; usually attached on a broad base; nearly spherical to more elongate; pitting regular and evident in all species except *B. ramosa*. Swarmers from resistant sporangia in *B. Pringsheimii* frequently give rise directly to asexual plants like their immediate parent. *Sexual reproduction* not yet clearly demonstrated in any species.

II. *Sphaerocladia* Stüben 1939

Characters of the family with exceptions noted above. Plants found on animal substrata.

Thallus unicellular, without pseudosepta or constrictions; sac-like, simple, ordinarily 60–140μ in diameter, or rarely elongate and slightly branched; attached to the substratum by rhizoids extending from all sides; usually being converted directly, in its entirety, into a single sporangium or gametangium.

Zoosporangia globose, with one to several discharge papillae. *Resistant sporangia* globose; outer heavy wall brown and smooth; germinating by a papilla which pushes through a tear in the outer wall.

Swarmers distinguished from those of related genera by the presence of a second "food body" lying adjacent to the nuclear cap.

Sexual reproduction by isoplanogametes; gametes of both sexes lacking pigment. Alternation of generations exactly as in *Euallomyces*.

III. *Blastocladiella* Matthews 1937, em. Harder and Sörgel 1938, 1939

Characters of the family. Plants known on both plant and animal substrata; rarely more than 1 mm. in height, often much smaller.

Thallus unicellular, without pseudosepta or constrictions; a simple or rarely slightly branched, globose to clavate or cylindrical hyphal tube anchored basally by rhizoids; bearing at its tip a single reproductive organ, i.e., either a zoosporangium, a resistant sporangium, or a gametangium.

Zoosporangia globose to cylindrical; usually with one, rarely up to three discharge papillae; not deciduous. *Resistant sporangia* globose or subspherical; not usually slipping from the outer hyphal sheath; outer heavy wall brown and with distinctive net-like sculpturing giving the appearance of irregular, shallow pits.

Sexual reproduction by isoplanogametes known in *B. variabilis;* gametes of one sex containing orange pigment. Alternation of generations in *B. variabilis* exactly as in *Euallomyces.*

IV. *Allomyces* Butler 1911, em. Kniep 1929, 1930, Emerson 1938

Syn. *Blastocladia* (as represented by *B. strangulata*) Barrett 1912.
Septocladia Coker and Grant 1922.

Characters of the family. Plants of all known species normally large and obvious, 1 cm. or more in height; forming loose tufts, similar in macroscopic appearance to members of the Saprolegniaceae; on both vegetable and animal substrata.

Thallus divided into segments by characteristic, incomplete, ring-like or wheel-shaped pseudosepta usually accompanied by more or less marked constrictions; basal part composed of several, often thick-walled segments arranged dichotomously and bearing progressively more slender fertile hyphae; branching regularly dichotomous, rarely subdichotomous or subumbellate, becoming clearly sympodial in the reproductive part. Basal segments 50–200×40–100μ more or less; hyphal segments 10–40μ in diameter and up to about 700μ in length.

Zoosporangia primarily terminal, formed on the hyphal tips and then becoming sympodially arranged; secondarily in chains, formed in basipetal succession and often in complex clusters; varying in shape from subspherical to ellipsoid, clavate, or nearly cylindric; with one to eight or more conspicuous exit papillae distributed over the surface and lacking a cone-shaped, inward projection; normally 40–135×20–55μ; not deciduous. *Resistant sporangia* also formed terminally and later becoming sympodially arranged; very rarely intercalary or in short chains; regular pitting usually evident in all known forms; 25–80×20–45μ. R.S. zoospores of *Cystogenes* encyst and function as small sporangia.

Sexual reproduction by anisoplanogametes in *Euallomyces.*

KEY TO THE SUBGENERA, SPECIES AND VARIETIES OF ALLOMYCES

A. Long life cycle, two equal generations; sexual plants bear female and male gametangia; resistant sporangia not usually deciduous, ordinarily with closely spaced pits.

Subgen. *Euallomyces*

1. Primary male gametangia hypogynous. *A. arbusculus.*
   a. Resistant sporangia averaging 32–45μ wide. var. *arbusculus.*
   b. Resistant sporangia averaging 24–31μ wide. var. *minor.*
2. Primary male gametangia epigynous. *A. javanicus.*
   a. Primary female gametangia not markedly longer than males; male and female gametangia in nearly equal numbers. var. *javanicus.*
   b. As (a) but with a marked preponderance (more than 90%) of male gametangia. var. *perandrus.*
   c. As (a) but with primary female gametangia usually 2 to 4 times longer than males. var. *macrogynus.*

B. Short life cycle, only one generation; R. S. zoospores germinate directly with a tube; resistant sporangia not usually deciduous, with closely spaced pits.

Subgen. *Brachyallomyces*

1. *A. anomalus.*

C. Short life cycle, only one obvious generation; R. S. zoospores encyst, function as small sporangia, and liberate secondary zoospores; resistant sporangia usually deciduous, with large, widely spaced pits.

Subgen. *Cystogenes*

1. Cytoplasm unpigmented; resistant sporangia all with broadly rounded apex. *A. cystogenus.*
   a. Resistant sporangia usually oval, averaging 51–55μ × 33–38μ. var. *cystogenus.*
   b. Resistant, sporangia usually elongate, clavate, averaging 71 × 35μ. var. *elongatus.*
2. Cytoplasm sometimes pigmented; resistant sporangia, about 70% with pointed apex. *A. moniliformis.*

Subgenus A. EUALLOMYCES Emerson 1938. With a long life cycle involving alternation of equal asexual and sexual generations. *Asexual plants* arising from zoospores (from thin-walled sporangia) or zygotes; bearing zoosporangia and resistant sporangia; characters of the genus. Resistant sporangia not usually deciduous, ordinarily with fine closely spaced pits. R.S. zoospores monoflagellate, germinating directly by a tube. *Sexual plants* arising from R.S. zoospores; similar to asexual stage in vegetative structure but bearing male and female gametangia in place of zoosporangia; hermaphroditic and self-fertile; sometimes producing resistant sporangia. Gametangia like zoosporangia in formation and arrangement; with one to several typical discharge papillae; often formed primarily in pairs, a male and a female together, but sometimes singly; secondarily in chains in basipetal succession; females unpigmented, gray; males strikingly pigmented with carotene, pale orange to brick-red, and often somewhat smaller than females. Gametes corresponding to zoospores in structure; females hardly distinguishable from the latter except by origin and behavior; males markedly smaller and more active, their lipoid granules containing orange pigment; males and females fusing in pairs to form biflagellate planozygotes which germinate immediately without a "rest" period.

In certain strains the R.S. zoospores, departing from the characteristic

developmental history just described, give rise more or less frequently directly to asexual plants, the sexual generation being omitted as in *Brachyallomyces*.

ALLOMYCES ARBUSCULUS Butler 1911, em. Barrett 1912 and Hatch 1933

Syn. *Blastocladia strangulata* Barrett 1912.
A. strangulata (Barrett) von Minden 1916.
Septocladia dichotoma Coker and Grant 1922.
A. arbuscula forma *dichotoma* (Coker and Grant) Kanouse 1927.
A. Kniepii Sörgel 1937.

Synonymy.—When Barrett (1912) described *Blastocladia strangulata* he was not aware of Butler's prior publication establishing the genus *Allomyces*. Nor were Coker and Grant (1922) when they described another isolate of *Allomyces* as *Septocladia dichotoma*. Von Minden (1916) suggested that *B. strangulata* which he renamed *Allomyces strangulatus* (Barrett) von Minden, and *A. arbusculus* Butler were probably identical. At any rate he saw clearly that they belonged in the same genus. Fitzpatrick (1923) concluded, moreover, from a critical comparison of texts and figures that there were no significant differences between *S. dichotoma* and *B. strangulata*, and Coker himself (1937) listed *B. strangulata* Barrett, *A. strangulatus* Minden, *S. dichotoma* Coker and Grant, and *A. arbusculus* forma *dichotomus* Kanouse as synonymous with *Allomyces arbusculus*. Finally, as the writer has explained (p. 108), the complete life cycles of all the isolates of *Allomyces* studied before 1929 were not definitely known, and therefore the four synonyms noted just above under *A. arbusculus* are included here on the assumption that these were long-cycle forms with hypogynous male gametangia and that the sexual stage was over-looked by the earlier investigators.

The problem of the synonymy of *A. Kniepii* Sörgel is obviously different since living material of the type isolate of this species is still available. Sörgel (1937) worked with four strains of *Allomyces* all of which formed hypogynous male gametangia. Three of these strains, one of which he obtained from the Centraalbureau voor Schimmelcultures at Baarn, Sörgel says (ff. p. 408) were all the same and were originally derived from the same isolate, Kniep's form from Bali. The fourth form which had been isolated by W. C. Coker from soil at Chapel Hill, North Carolina, Sörgel obtained from Miss Kanouse, labelled *A. arbusculus*. Sörgel (1937) gave the Bali form a new specific name, *A. Kniepii*, on the following criteria:

A. Kniepii . . . Bali form	*A. arbusculus* . . . from Miss Kanouse
1. male gametangia always hypogynous	male gametangia sometimes epigynous
2. resistant sporangia larger and truncate	resistant sporangia smaller and not truncate
3. growth rate slower	growth rate more rapid

Measurements in support of the last two differences were not given, nor were counts made to show the frequency of epigyny in *A. arbusculus*. The writer has had Miss Kanouse's strain of *Allomyces* in culture since 1934 (isolate North Carolina 1) and obtained the Bali strain from the Centraalbureau voor Schimmelcultures at Baarn in 1937 (isolate Bali 1). He has been able therefore to compare these two strains carefully with each other as well as with the other hypogynous forms in his collection.

From the preceding section dealing with the problem of specific and varietal differences it will be evident that the morphological characters with which Sörgel has sought to distinguish *A. Kniepii* intergrade so fully that they can not, in the writer's opinion, be used satisfactorily to delimit species. The writer has never found, in any of the isolates of *A. arbusculus* (including North Carolina 1), that epigynous male gametangia are sufficiently prevalent to be distinctive: in all these isolates primary pairs of female and male gametangia with the male in the terminal position are very rare, and certainly epigyny in *A. arbusculus* is far less frequent than is hypogyny in certain isolates (Java 1, Tanganyika 3A) of *A. javanicus*. It is true that there is, as Sörgel has noted, quite a marked difference between the average size of resistant sporangia of North Carolina 1, Sörgel's *A. arbusculus*, and Bali 1, Sörgel's *A. Kniepii*. The relation between the sizes of these isolates is shown in Fig. 9 and Table 4. Yet, even if the intergradation in the size of resistant sporangia throughout the group were not so gradual, it would still not be valid to consider the form with smaller resistant sporangia as typical of *A. arbusculus* and give a new specific name, as Sörgel has done, to the form with larger resistant sporangia, for the type isolate of *A. arbusculus*

(India 1) had even larger ones. Butler's own measurements of the type material of *A. arbusculus* (1911, p. 1027; 40–60μ × 30–45μ) show that the forms with larger resistant sporangia should be considered as typical of the species. The isolate studied by Hatch, North Carolina 2, also falls in this larger, typical group. The writer has, therefore, included all the forms, such as Bali 1, India 1, and North Carolina 2, which have larger resistant sporangia in the type variety of *A. arbusculus* and placed forms such as North Carolina 1 with smaller resistant sporangia in the new variety *minor*.

Characters of the subgenus. Primary paired gametangia with the male characteristically subterminal in position (hypogynous), the female terminal. Zoosporangia oval or barrel-shaped, tending to become subspherical with age or in solid media; normally 40–70×30–40μ. Zoospores, mean diameter 9–12μ. Resistant sporangia always produced in abundance; nearly oval, 20–81×16–60μ; normally tawny- to reddish-brown; pits distinct and averaging 1μ apart. R.S. zoospores, mean diameter 7.5–10.5μ. Gametangia primarily regularly paired, secondarily in short chains; females strikingly globose or subspherical, especially when older; males somewhat smaller, usually barrel-shaped when young, becoming more nearly spherical with age, very rarely occurring terminally (epigynous) in the primary pairs. Female gametes, mean diameter 8–11.5μ; male gametes 5.5–8.5μ.

Var.[1] **arbusculus** var. nov.[2] With resistant sporangia averaging 32–45μ wide.

Var.[1] **minor** var. nov.[2] With resistant sporangia averaging 24–31μ wide.

Allomyces javanicus Kniep 1929

Characters of the subgenus. Primary paired gametangia with the male characteristically terminal in position (epigynous), the female subterminal. Zoosporangia ellipsoid or barrel-shaped, seldom becoming subspherical; normally 40–85×25–50μ. Zoospores, mean diameter 10–12μ. Resistant sporangia subspherical to oval, 35–75×25–60μ; olivaceous brown to tawny or reddish-brown; pits about 1μ apart, usually distinct but sometimes very fine or almost indistinguishable. R.S. zoospores, mean diameter 9–11μ.

Var. **javanicus** var. nov. Gametangia usually rather irregular in shape and arrangement, tending to occur singly or in chains; primary males sometimes hypogynous; females often only slightly longer than males in the primary pairs (averaging about 1.5 times as long as males); females and males usually produced in approximately equal numbers. Female gametes, mean diameter 9–11μ; male gametes 4–5.5μ. Resistant sporangia formed in abundance or, in certain isolates, sparsely except under adverse conditions; sometimes gray or olivaceous brown; usually with evident pits.

[1] A complete series of intergrades occurs; hence this varietal separation is arbitrary and mainly for convenience. See p. 120.

[2] Latin descriptions of new varieties and species have been placed at the end of the Taxonomic Outline (p. 134).

Var. **perandrus** var. nov. As above but with a great preponderance of male gametangia; about 95% male to 5% female.

Var. **macrogynus** (Emerson and Fox) comb. nov.[3]

Syn. *A. macrogynus* Emerson and Fox 1940

Gametangia, particularly on young hyphae, regular in shape and arrangement; primary and often secondary ones very regularly paired; primary males always terminal; females markedly elongate in the primary pairs, often nearly cylindric, strikingly longer than males (averaging about 2 times and not seldom 3 to 4 times as long). Female gametes, mean diameter 9–12μ; male gametes 4–6μ. Resistant sporangia always formed in abundance even on young hyphae; pits usually very fine, sometimes almost indistinguishable.

Subgenus B. Brachyallomyces Emerson 1939. With a short life cycle lacking alternation of generations and sexual stage. Plants arising from zoospores or directly from R.S. zoospores; bearing zoosporangia and resistant sporangia; morphologically similar in all respects to the asexual stage of *A. arbusculus* var. *arbusculus*. Resistant sporangia not usually deciduous, with fine, closely spaced pits. R.S. zoospores monoflagellate, germinating directly by a tube.

Allomyces anomalus sp. nov.

Characters of the subgenus. (A problematical subgenus and species tentatively established to include those few isolates in which repeated attempts to obtain sexual plants have been unsuccessful. See p. 108.)

Subgenus C. Cystogenes Emerson 1938. With a short life cycle of only one obvious generation, but clearly distinguished from all other forms by the regular encystment of the primary R.S. zoospores immediately after their emergence. Primary R.S. zoospores usually biflagellate; sluggish, without any true swarm period. Cysts spherical, sometimes variable in size, 9–15μ in diameter, each forming a single discharge papilla and functioning as a little sporangium; discharging small secondary R.S. zoospores, usually in groups of four. Secondary R.S. zoospores (from cysts) mono-

[3] While the present paper was in press I read with great interest Indoh's report of Blastocladiaceae collected in Japan. (Indoh, H., 1940. Studies on Japanese aquatic fungi, II. The Blastocladiaceae. Science Reports of the Tokyo Bunrika Daigaku, Sec. B, **4**: 237–283.) From Indoh's figures and detailed descriptions it is apparent that: (1) *Allomyces javanicus* var. *macrogynus* n. comb. is nearly identical to *A. javanicus* var. *japonensis* Indoh, and (2) *A. cystogenus* sp. nov. is, as Indoh (loc. cit. p. 273) has suggested, closely similar to *A. neo-moniliformis* Indoh. Certain measurements however and other characters which I have described are not discussed by Indoh, and hence it seems best to omit any final analysis of the similarities and differences between his strains and my own until direct comparison of the living isolates has been made.

flagellate; with a definite swarm period; mean diameter 6–9μ or sometimes larger due to non-cleavage.

Plants arising from zoospores or directly from secondary R.S. zoospores; always bearing zoosporangia and resistant sporangia. Zoosporangia characteristically more elongate than in other subgenera; ellipsoid to clavate. Resistant sporangia also sometimes elongate; usually a pale yellow-brown; at maturity being shed in great numbers, usually by slipping from the enclosing hyphal sheath; pits very evident, more widely and less regularly distributed, averaging about 2μ apart. One species forming carotenoid pigment in the sporangia and hyphae under certain conditions.

Allomyces cystogenus sp. nov.[3]

Characters of the subgenus. Zoosporangia somewhat elongate, 50–120 × 20–40μ; secondary ones in longer or shorter chains. Zoospores, mean diameter 10–12μ. Resistant sporangia oval to very elongate and almost clavate but always with broadly rounded apex, 34–95 × 24–49μ. Primary R.S. zoospores and cysts quite constant in size; mean diameter 9–12μ. Secondary R.S. zoospores, mean diameter 6–7μ. Plants never producing pigment in the cytoplasm as far as known.

Var. **cystogenus** var. nov. Resistant sporangia oval, less elongate, averaging 51–55μ × 33–38μ; frequently germinating rapidly and with great uniformity as to time of germination and size of cysts produced.

Var. **elongatus** var. nov. Resistant sporangia strikingly more elongate than those of any other form; sometimes clavate; averaging 71 × 35μ; not usually germinating readily or uniformly.

ALLOMYCES MONILIFORMIS Coker and Braxton 1926, em. Emerson 1938

Characters of the subgenus. Primary zoosporangia often markedly elongate (2 to 5 times as long as broad); 50–135μ × 20–35μ. Secondary ones sometimes in long chains, 20 to 30 in a straight row in old cultures. Zoospores, mean diameter 9–12μ. Resistant sporangia elongate, ellipsoid and usually (about 70%) bluntly pointed at the tip; 38–87μ × 22–39μ, averaging 53–63μ × 30–35μ. Primary R.S. zoospores and cysts 11–15μ in diameter (sometimes up to 20μ, probably due to non cleavage). Secondary R.S. zoospores (from cysts) mean diameter 8–9μ. Plants on oatmeal agar or in old water-cultures accumulating conspicuous quantities of carotene distributed unevenly in oil droplets within the cytoplasm of hyphae, zoosporangia, and resistant sporangia.

Latin Diagnoses[4]

Although the writer (1938 and 1939) described the essential character-

[3] See p. 133.

[4] I am particularly grateful to Dr. David H. Linder for preparing these Latin descriptions.

istics of the three subgenera of *Allomyces* in earlier papers, Latin descriptions were not given at that time and have therefore been included here with the diagnoses of new species and varieties.

A. Subgen. EUALLOMYCES Emerson 1938

Cyclus vitae longus et vices aetatis stato aequo sexuali asexualique; thalli asexuales zoosporiis vel "zygotes" enati et zoosporangia sporangiaque perdurantia producentes; thalli sexuales hermaphroditici zoosporis ex sporangiis perdurantibus enati et gametangia masculina et femina parientes; sporangia perdurantia rare decidua, parietibus dense et parve lacunosis; zoosporae sporangiorum perdurantium uniflagellatae et tubulo germinantes.

B. Subgen. BRACHYALLOMYCES Emerson 1939

Cyclus vitae brevis et vices aetatis statusque sexualis absunt; thalli, zoosporis vel zoosporis ex sporangiis perdurantibus enati, zoosporangia et sporangia perdurantia parientes; sporangia perdurantia rare decidua parietibusque dense et parve lacunosis; zoosporae sporangiorum perdurantium uniflagellatae et tubulo germinantes.

C. Subgen. CYSTOGENES Emerson 1938

Cyclus vitae brevis et vices aetatis absunt; thalli zoosporis vel zoosporis secundariis ex sporis immotis (cysts) enati zoosporangiaque et sporangia perdurantia parientes; sporangia perdurantia typice decidua parietibusque laxe et grande lacunosis; zoosporae primariae sporangiorum perdurantium frequenter biflagellatae sunt, non tubulo germinantes sed semper in sporas immotas convertentes; sporae immotae sporangiorum parvorum vice funguntur et zoosporas secundarias uniflagellatas liberant.

A. javanicus var. **javanicus** var. nov.

Gametangia saepe irregulari forma dispositioneque sunt; gametangia primaria femina non notate gametangiis masculinis longiora; sporangia perdurantia nonnumquam non numerosa sed semper parietibus conspicue lacunosis.

A. javanicus var. **perandrus** var. nov.

Typo similis est sed gametangia masculina multo preponderant (90% plus).

A. javanicus var. **macrogynus** var. nov.

Gametangia hypharum juvenilium plerumque forma dispositioneque stabilia sunt; gametangia primaria femina notate elongato-cylindrica, typice gametangiis masculinis bis vel quater longiores; sporangia perdurantia semper numerosa et typice parietibus parvissime lacunosis.

A. arbusculus var. **arbusculus** var. nov.

Sporangia perdurantia 32–45μ diametro medio.

A. arbusculus var. **minor** var. nov.

Typo similis est sed sporangia perdurantia 24–31μ diametro medio.

A. anomalus sp. nov.

Cyclus vitae brevis et vices aetatis statusque sexualis absunt; thalli, ex zoosporis sporangiorum perdurantium, semper zoosporangia et sporangia perdurantia parientes quae stato asexuali *Allomycetis arbusculi* var. *arbusculi* similia sunt; zoosporae 9–12μ diametro; sporangia perdurantia 34–87×24–55μ, ovalia, apice rotundato, typice non decidua parietibusque dense et parve lacunosis; zoosporae sporangiorum perdurantium uniflagellatae, 8–10μ diametro.

A. cystogenus sp. nov.

Cyclus vitae brevis et vices aetatis absunt; thalli zoosporis vel zoosporis secundariis ex sporis immotis (cysts) enati et zoosporangia sporangiaque perdurantia parientes; zoosporangia typice 50–120×20–40μ; zoosporae 10–12μ diametro medio; sporangia perdurantia ovata vel clavata, semper apice late rotundato parietibusque laxe et grande lacunosis, typice decidua; zoosporae primariae sporangiorum perdurantium 9–12μ diametro, frequenter biflagellatae sunt et semper in sporas immotas sine tempore natationis convertunt, non tubulo germinantes; sporae immotae 9–12μ diametro sporangiorum parvorum vice funguntur et per papillam emissionis unicam zoosporas plerumque quattuor secundarias uniflagellatas liberant; zoosporae secundariae 6–7μ diametro medio et tubulo germinantes.

var. **cystogenus** var. nov.

Sporangia perdurantia ovalia, 34–95×24–45μ, typice 51–56×33–38μ.

var. **elongatus** var. nov.

Typo similis est sed sporangia perdurantia elongato-ovalia vel clavata, 54–85×24–41μ, typice 71×35μ.

General Discussion

VARIABILITY AND INTERGRADATION: RETICULATE PATTERN OF CHARACTERS

Copulation of uninucleate cells is rare among the Phycomycetes. To be sure, fusion of isogametes has been observed in a few species of Archimycetes, but *Blastocladiella*, *Allomyces* and *Monoblepharis* are, as far as we know at present, the only genera of filamentous Phycomycetes in which

uninucleate gametes are produced. Sparrow (1933) has emphasized the variability and intergradation between species of *Monoblepharis*, and it should be evident from the foregoing analysis of characters in *Allomyces* that a similar situation exists in that genus. This variation is very possibly a result, at least in part, of interspecific and intervarietal hybridization. The writer has found that well defined epigynous and hypogynous species of *Allomyces* can readily be crossed in culture, and there is no *a priori* reason to suppose that hybridization does not occur in nature. Actually, given motile gametes and different forms growing, as they do, in the same localities, *A. arbusculus*, Burma 1Db, and *A. javanicus*, Burma 1Da, for example were isolated from the same sample of soil, crossing in nature seems very probable. F_1 hybrids obtained in the laboratory are sometimes intermediate between their parents and clearly illustrate the difficulties attendant upon an attempt to establish clear-cut species or varieties. While the evolution of the present forms of *Allomyces* has very probably resulted in part from various sorts of mutation, it seems probable that natural hybridization has also played an important role in the process. Bearing this in mind we may fully expect to find, just as we do in fact, that isolates do not always fall in neat clear-cut compartments, but on the contrary show intergradation; forms with one or more intermediate characters usually appear if a sufficiently large population is studied in detail. Furthermore, the different characters do not often show a parallel transition: that is to say, if we arrange a series of forms such as is shown in Fig. 9 on the basis of resistant sporangial size and another on the basis of some other character such as pigmentation, or etc., the two series do not necessarily agree at all. There is, as one would expect, a reticulate rather than a parallel grouping of varying characters; each isolate has its own particular set of individual peculiarities. If as each new isolate is found it is designated as a new variety or even species on the basis of the small differences between it and one or two other forms, one soon finds that every grade of intermediate has a separate name and they all overlap to such an extent that they can no longer be named with certainty. If on the other hand specific distinctions are made with relatively sharp, non-variable characters and are at the same time sufficiently inclusive, it is no longer so difficult to fit new isolates into their proper species or even variety.

The problem of intergrading species and even genera in other families of the water moulds, particularly the Saprolegniaceae, has become a major one. Similar problems have been encountered in many groups of Phanerogams, as well as Cryptogams. In a recent paper on the species concept in *Fusarium*, for example, Snyder and Hansen (1940) concluded from a study of single spore cultures that many supposedly valid species overlap to such an extent that they can not be separately distinguished and must be reduced to synonymy. It is the writer's hope that those who carry out future

taxonomic investigations in *Allomyces* may refrain from describing new species on the basis of varying and intergrading characters, at least until they have made a detailed study of such characters in single spore strains and compared them in a large number of natural isolates.

GEOGRAPHICAL DISTRIBUTION—FREQUENCY OF OCCURRENCE

From the fairly large number of isolations which have been made during the last ten or fifteen years it is possible to say with certainty that *Allomyces* is confined to the tropical and warmer temperate regions. In the earlier collections it was rarely found north of 40° N. or south of 40° S. latitude. It appeared only three times in Harvey's numerous Wisconsin soil collections (1928); it was found once in Ithaca, New York, and once (?) in Illinois by Barrett. In spite of intensive collecting it has not yet been discovered in the region around Cambridge, Massachusetts, and although it may possibly exist here it is certainly not common. Of twenty-four soil samples collected in Portugal, one from the vicinity of Montedor yielded an isolate of *A. arbusculus* in August, 1939. As far as the writer is aware this is the only isolate which has been found in Europe up to the present time. The writer has tried a small number of soil samples from southwestern Ireland and central Italy without discovering *Allomyces*, but future collections around the Mediterranean basin will almost certainly reveal members of the genus in that region, and some may also be found in the southern parts of the British Isles.

We may contrast the apparently rare occurrence of *Allomyces* in cold-temperature zones with its frequent occurrence in warmer latitudes. In the past fifteen years species of the genus have repeatedly been collected in North Carolina and other southern states. *A. arbusculus* was first discovered in India; Kniep's four or more forms came from Java and Bali; Wolf (1939) has shown that *Allomyces* occurs commonly in Mexico. Recently Sparrow, Salvin, Wolf and others have obtained many additional isolates which show that *Allomyces* can be found in many parts of Central America and northern South America. The writer's own isolations are particularly indicative of a very general, world wide tropical distribution. Of the 115 odd soil samples sent from tropical and warm-temperate regions of both hemispheres, more than twenty-five per cent have yielded isolates of *Allomyces*. Seven separate collections of earth from various sites in Rangoon, Burma, all proved to contain resistant sporangia of the fungus, for isolations were made from every one. The accompanying maps, Fig. 16, show how widely the genus is distributed, and further investigations will almost certainly reveal that it is even more cosmopolitan. *A. arbusculus* has been isolated most frequently and appears to be more generally widespread than any of the other species. Within the past year isolates of

A. javanicus have been obtained for the first time in the western hemisphere and identified as such. Wolf's isolate, Mexico 17, Sparrow's Texas 2, and a third form isolated from soil from Monument Valley, Arizona, by

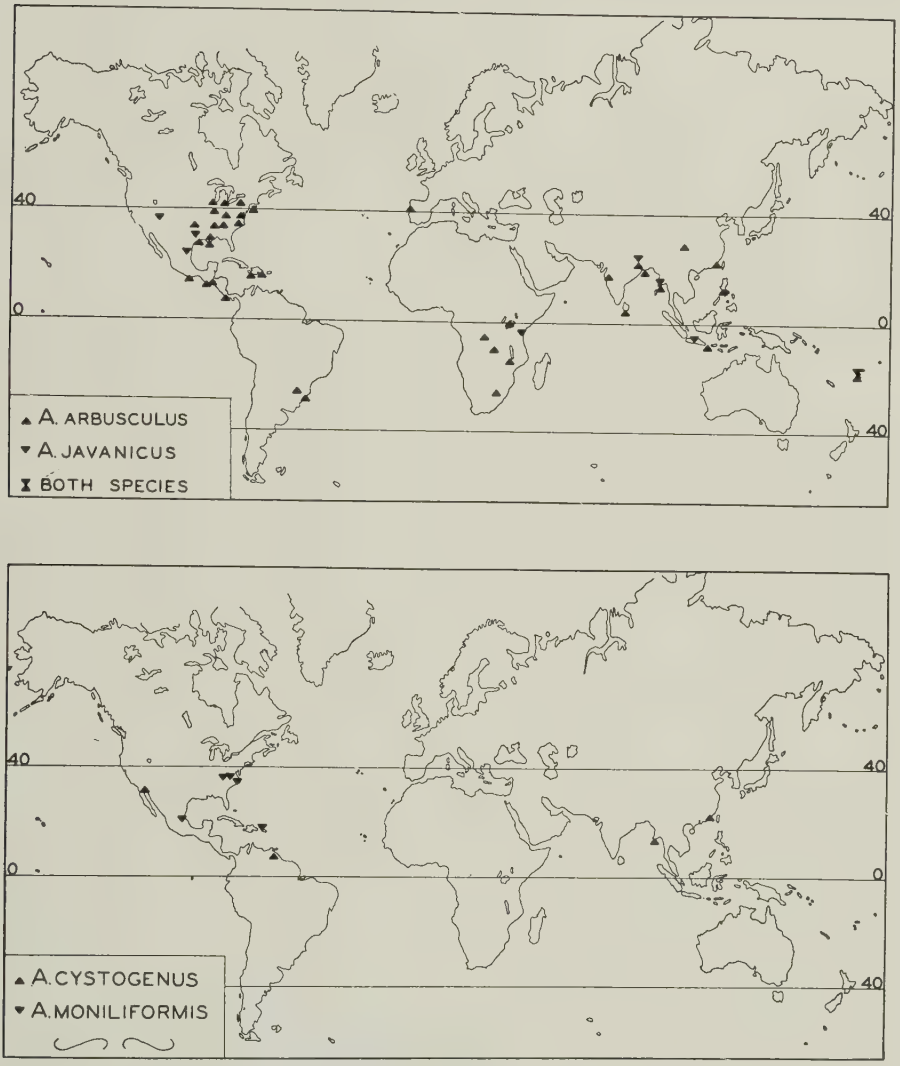

FIG. 16. Maps showing the localities where *Allomyces* has been collected: above, *Euallomyces;* below, *Cystogenes*. For the three isolates of *Brachyallomyces* see p. 84.

Harvey and studied by Wolf all produce epigynous male gametangia and are strains of *A. javanicus*. The cyst producing types are apparently relatively rare.

RELATIONS AND POSSIBLE ORIGIN OF THE GENUS

Two theories, basically opposed to each other, have been put forward in attempts to explain the origin of the filamentous Phycomycetes, one deriving them from chlorophycean forms, the other postulating certain chytridiaceous ancestors. Without entering into a detailed account of this argument, which has continued for nearly a century, we may note very briefly here the main trends which have led to our present understanding of the relations of the Phycomycetes, particularly the aquatic forms. In the latter eighteen hundreds and even in the early part of the present century many botanists (A. Braun, Pringsheim, Sachs, Charles Bessey, et al.) firmly adhered to the belief that the fungi had evolved directly from the various groups of algae by loss of chlorophyll and assumption of a saprophytic or parasitic way of life. Yet, as a result of the work of Bohlin, Luther, Blackman and Tansley, and others, the fundamental significance of zoospore structure and flagellation in determining algal relations was being clearly recognized, and it was becoming evident that the filamentous Chlorophyceae had probably evolved from flagellate ancestors. So too, as more detailed information about the swarmers and reproductive processes in the Phycomycetes was being gained, it no longer seemed valid to base relations between these fungi and the algae on gross morphological features. Many investigators were coming to look upon the fungi as a distinct class quite separate from the algae. As early as 1882 J. Klein suggested that the phycomycetous fungi had evolved in a progressive series of their own from simpler to more complex forms. Since then students of the Phycomycetes have accepted this idea ever more fully as knowledge of new forms and detailed understanding of their structure and methods of reproduction have steadily increased. Lotsy (1907, p. 110) stressed the significance of the flagella of the zoospores of the aquatic fungi, pointing out that there are two distinct lines, the biflagellate and the monoflagellate, and indicating the fallacy in deriving the monoflagellate forms from isokont green algae. In his detailed analysis of relations in the Phycomycetes Atkinson (1909) further stressed the striking differences in the formation, structure, and behavior of the zoospores in this group and the motile reproductive cells in the Chlorophyceae. More recently Scherffel (1925), Sparrow (1935a and b), and Weston (1935) have clearly set forth all the lines of evidence which lead us to believe now that the filamentous Phycomycetes have probably evolved from lower fungi rather than from green algae.

Allomyces resembles in its gross structure certain branching, filamentous Chlorophyceae such as *Cladophora* or *Dichotomosiphon* and in its sexual reproduction by anisoplanogametes is similar to such forms as *Bryopsis*, *Codium*, etc. Relations with the green algae can not, however, as has just been pointed out, be soundly based on superficial resemblances of thallus form or general similarities of reproductive processes. Closer comparison

reveals marked differences, and here perhaps the most fundamental of these is in the morphology of the swarmers. Petersen (1910), appreciating the phylogenetic significance of the monoflagellate zoospores in *Blastocladia*, removed this genus from the biflagellate Leptomitales and erected the order Blastocladiales. From the studies of Barrett (1912), Kniep (1929) and others it has become evident that the motile reproductive cells of the Blastocladiales are strikingly similar to those of the Monoblepharidales: all are characterized by a single, posterior flagellum and a central, top-shaped nuclear cap or food-body. Sparrow (1933) suggested that both families might well be placed in a single order primarily on this basis. At any rate it is clear that a much closer relation exists between these two groups than can be shown between the Blastocladiales and any of the algae.

Moreover, as many people have pointed out (Scherffel 1925, Weston 1935, et al.), the motile cells of the Monoblepharidales and Blastocladiales show great similarity to the monoflagellate swarmers of the true chytridiaceous forms. Now one can, by gradual transitions, trace the evolution of sexuality in the Chlorophyceae from isogamy through anisogamy to highly developed oogamy. That a complete series of ascending forms of this sort was not known in the aquatic Phycomycetes has, as Kniep (1929) so clearly showed, appeared to favor the derivation of the oomycetes from oogamous algae rather than lower fungi. Kniep's discovery (1929) of anisogamous planogametes in *Allomyces* was obviously of extreme significance in this connection, and Weston (1935), after pointing out the relation which exists between *Monoblepharis* on the one hand and the Chytridiales on the other, made the suggestion that future investigations would almost certainly reveal the existence of monoflagellate, isogamous Phycomycetes intermediate in thallus complexity between the true chytrids and *Allomyces*. This prophecy came true only three years later when Harder and Sörgel (1938) discovered isogamous, planogametic reproduction in Miss Matthews' genus *Blastocladiella*, and it has been further borne out recently by Stüben's (1939) discovery of *Sphaerocladia*, another genus of the Blastocladiales with a very simple thallus and isoplanogametes. In *Blastocladiella variabilis* the gametes are equal in size but those of one sex have an orange pigment which clearly relates them to the male gametes of *Euallomyces*, whereas in *Sphaerocladia variabilis* the gametes of both sexes are unpigmented and more truly isogamous.

There is now, therefore, very good reason to suppose that both *Allomyces* and *Monoblepharis* evolved from lower fungi with monoflagellate swarmers and isogamous planogametes. Indeed the life cycle of *Blastocladiella variabilis* is so nearly identical in all respects with that of *Euallomyces* that we may go so far as to say that *B. variabilis* very probably represents the ancestral form of such species as *Allomyces arbusculus* and *A. javanicus*.

Blastocladiella simplex Matthews (1937) apparently lacks a sexual generation and is closely comparable in life cycle with *Allomyces anomalus* n. sp. of *Brachyallomyces*. No simple blastocladiaceous forms with a life cycle similar to that of *Cystogenes* are yet known, but Chytrid-like organisms which produce cysts in this way will very possibly be found at some future time. Perhaps future investigation will also reveal isogamy or anisogamy in some or all of the eleven species of *Blastocladia* which are now known. Miss Blackwell has shown (1940), however, that in *B. Pringsheimii* there is a life cycle which, since it apparently lacks a sexual stage, is exactly similar to that of *Brachyallomyces*, and at present there is no conclusive evidence of sexuality in *Blastocladia*.

The following tabular comparison of the genera and species in the Blastocladiales will serve to summarize our present knowledge of the life cycles and types of sexuality in the order.

TABLE 6. Comparison of Life Cycles in the Four Genera of the Blastocladiales.

GENUS	TYPES OF LIFE CYCLES		
	I. Euallomyces	II. Brachyallomyces	III. Cystogenes
1. ALLOMYCES	*A. arbusculus* *A. javanicus* (anisogamous)	*A. anomalus*	*A. cystogenus* *A. moniliformis*
2. BLASTOCLADIELLA	*B. variabilis* (isogamous except in pigmentation)	*B. simplex*	.....
3. SPHAEROCLADIA	*S. variabilis* (isogamous)	.....	.....
4. BLASTOCLADIA	.....	*B. Pringsheimii*	.....

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The Comparative Anatomy of the Secondary Xylem of Four Oriental Species of Celtis

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The study of wood anatomy assists taxonomists materially in placing plants of doubtful position into schemes of classification, which have hitherto been based primarily on external morphology and floral structure (3, 7, 10). From this point of view the present investigation deals with the wood anatomy of certain Oriental species of Celtis (Ulmaceae), e.g., their specific anatomical characters and the variation of these. Celtis, commonly called hackberry, is a genus composed entirely of large, or medium-sized, trees and of shrubs. The genus is of ancient origin, and is distributed over temperate and tropical regions of both eastern and western hemispheres (1). According to Berry (1) the genus consists of 80 to 100 species. In this study the four species examined were *C. sinensis* Pers., *C. philippinensis* Blanco., *C. tetrandra* Roxb., and *C. crenata* Planch.

The material was prepared according to the technique outlined by Wetmore (12) and eight to twelve slides were made for each of the eight specimens studied. Measurements were made to secure the average vessel diameter (tangential) of each species. To obtain these, 25 vessels from each specimen, selected at random from the transverse sections, were measured and the measurements averaged. In the case of ring-porous woods, 25 vessel diameters in the early wood and 25 in the late wood were measured and averaged.

In order to measure more accurately vessel and fiber length, bits of wood from each specimen were cut into sections about 300μ thick and macerated according to Jeffrey's method (2). The lengths of 50 fibers and of 50 vessels for each specimen were measured, and the average for fibers and for vessel elements calculated.

The terms used in describing the woods of the four species are those approved by the Committee on Nomenclature of the International Association of Wood Anatomists (4), as amended and illustrated by Record (9). Kribs' (5) ray classification was adopted in this study.

ANATOMICAL DESCRIPTION OF SPECIES

Celtis sinensis

C. sinensis Pers., also called *C. chinensis* Bunge, and *C. japonica* Planch., is native to China, Japan, Korea, and Formosa (8) (11). Four different specimens (H9046, H5174, H9045, and H9050) were examined.

[1] The writer wishes to express her gratitude to Dr. Oswald Tippo, under whose supervision this investigation was carried on, for suggesting the problem, for securing the wood materials, and for his kindness in reading the manuscript. She wishes to thank Professor R. H. Wetmore of Harvard University for donating the wood.

Growth rings are present in all specimens. They vary from 564μ to 6970μ in width.

Fibers are of the libriform type, thick (lumen less than thickness of wall) to very thick (lumen almost closed) walled, gelatinous (Fig. 4), and with small, rounded, simple pits. Lengths vary from 349 to 1477μ (av. 923μ; mostly 644–1242μ).

Vessels, varying from 15 to 99 per sq. mm., are solitary (30 to 85%), in multiples of 2 to 4 (few to 33%), or in clusters of 2 to 35 (10 to 70%). The pores are rounded to angular. Diameters in early wood vary from 116 to 298μ (av. 218μ; mostly 133 to 282μ) and those in late wood from 17 to 83μ (av. 46μ; mostly 20 to 66μ). Walls of vessel elements are thin (less than 5μ) and lengths of elements measure from 83 to 349μ (av. 231μ; mostly 197 to 299μ). Perforations are simple, and walls oblique (25°) to transverse; intervascular pits numerous, medium-sized (7 to 10μ in diameter) rounded, and alternate. Vessel-parenchyma pits are alternate. In all specimens, except H5174 (a two-year-old stem), there are few to numerous tyloses with thin walls and simple pits. Many of the vessels, in all specimens except the two-year-old stem, have spiral thickenings. In all cases the wood is ring-porous (Fig. 1).

Rays, averaging 6 to 12 per mm., are both uniseriate and multiseriate. The width of multiseriate rays varies from 2 to 14 cells (mostly 3 to 9). The height of uniseriate rays numbers from 2 to 18 cells (mostly 4 to 11); that of the multiseriates, from 8 to 127 cells (mostly 35 to 95). Pits to contiguous vessels and to other ray cells are small, numerous, and simple. Rhomboid crystals are present in all cases, except in the young stem. The type of ray varies from heterogeneous II A to heterogeneous II B (found only in H9045).

Cells of parenchyma are abundant and simple-pitted. The type of parenchyma distribution is terminal (one to several rows), vasicentric and confluent.

There is little variation among the four specimens. One (H9050) has rays somewhat deeper than those in the other specimens, and sheath cells around the multiseriate rays. In this same specimen pores are more numerous to the sq. mm. than in any of the others. Ray type differs in one specimen. All are heterogeneous II A except H9045, which is heterogeneous II B.

Celtis philippinensis

C. philippinensis Blanco., also known as *C. strychnoides* Planch., is native to the Philippine Islands, to certain islands off the coast of southeastern China, and to French Indo-China (6). Two specimens (H18098 and H5370) were examined.

No growth rings are found with the exception of a few traces in H18098.

Fibers are libriform, thin (lumen greater than thickness of wall) to very thick (lumen almost completely closed) walled, gelatinous, and with small,

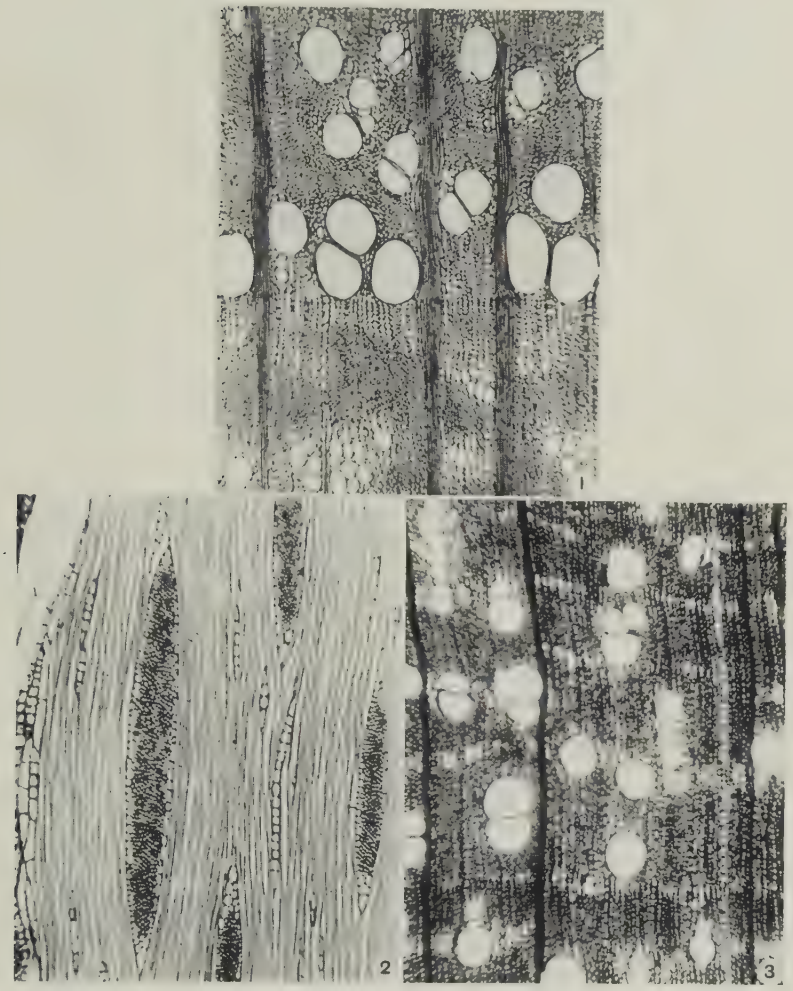

FIG. 1. *Celtis sinensis* Pers. (H9046). Transverse section (×70), showing ring-porous wood with growth rings and portions of early wood and late wood; pores solitary, in multiples, or in clusters; parenchyma terminal (2 to 8 seriate), vasicentric, and confluent; libriform wood fibers; and multiseriate rays.

FIG. 2. *Celtis tetrandra* Roxb. (H9047). Tangential section (×70), showing uniseriate and multiseriate heterogeneous rays; tall multiseriate ray, with sheath cells; and portion of a vessel to the left.

FIG. 3. *Celtis crenata* Planch. (H9040). Transverse section (×70), showing diffuse-porous wood with traces of growth rings; pores mostly solitary or in multiples; parenchyma terminal (2–5 seriate), metratracheal, vasicentric, aliform, and confluent; libriform wood fibers; and narrow multiseriate rays.

rounded, simple pits. Fiber lengths measure from 365 to 1394μ (av. 1032μ; mostly 863 to 1328μ).

Vessels, averaging 11 per sq. mm. in one specimen (H18098) and 23 in the other (H5370), are solitary (80%) or in multiples of 2 to 3 (20%). Pores are rounded, the diameters varying from 83 to 216μ (av. 156μ; mostly 133 to 183μ). Walls of vessel elements are thin (less than 5μ) and the lengths of elements vary from 149 to 564μ (av. 380μ; mostly 282–481μ). Perforations are simple, end walls oblique (35°) to almost transverse (80°); and intervascular pits are abundant, medium-sized (7 to 10μ in diameter), rounded, and alternate. Vessel-parenchyma pits are alternate. None of the vessel elements have spiral thickenings. Tyloses with fairly thick walls and simple pits are numerous in H5370 but are not found in 18098. The wood is diffuse-porous.

Rays, 9 to 10 per mm., are uniseriate and multiseriate. The width of the multiseriate rays varies from 2 to 8 cells (mostly 4 to 6). The height of the uniseriate rays varies from 2 to 24 cells (mostly 6 to 13) and that of the multiseriate rays from 15 to 47 cells (mostly 20 to 30). Pits to vessels are small, numerous, and simple. Crystals are present in ray cells. Rays are heterogeneous I (Fig. 5), almost heterogeneous II A.

Parenchyma cells with small, simple pits are abundant. Distribution is paratracheal—in one case (H18098) mostly aliform with a small amount of confluent and in the other (H5370), mostly confluent with a small amount of aliform.

Variation among the specimens is slight. One specimen (H5370) has thick-walled tyloses and pores somewhat smaller and more numerous than those of the other specimen.

Comparison with other species.—In contrasting the woods of *C. sinensis* and *C. philippinensis* a few differences can be mentioned. The former is ring-porous and has growth rings, while the latter is diffuse-porous and has only traces of growth rings. *C. sinensis* has more pores per sq. mm. than *C. philippinensis*. In cross-section, the pores of *C. sinensis* are rounded to angular, those in *C. philippinensis* are mostly rounded. *C. sinensis* has, on the average, shorter fibers and shorter vessel elements than *C. philippinensis*. End walls of vessel elements in *C. sinensis* are oblique to transverse; those in *C. philippinensis* oblique to almost transverse. Spiral thickenings are found in many vessel elements in *C. sinensis*, but none in those of *C. philippinensis*. Rays in *C. cinensis* are heterogeneous II A or II B; those in *C. philippinensis* heterogeneous I, almost II A. Multiseriate rays in the former are several cells wider and much taller than in the latter. One specimen of *C. sinensis* (H9050) has sheath cells. Parenchyma distribution is paratracheal in both woods. In the first, vasicentric and confluent; in the latter, mostly aliform and confluent. *C. sinensis* has one to several rows of terminal parenchyma in addition to the paratracheal

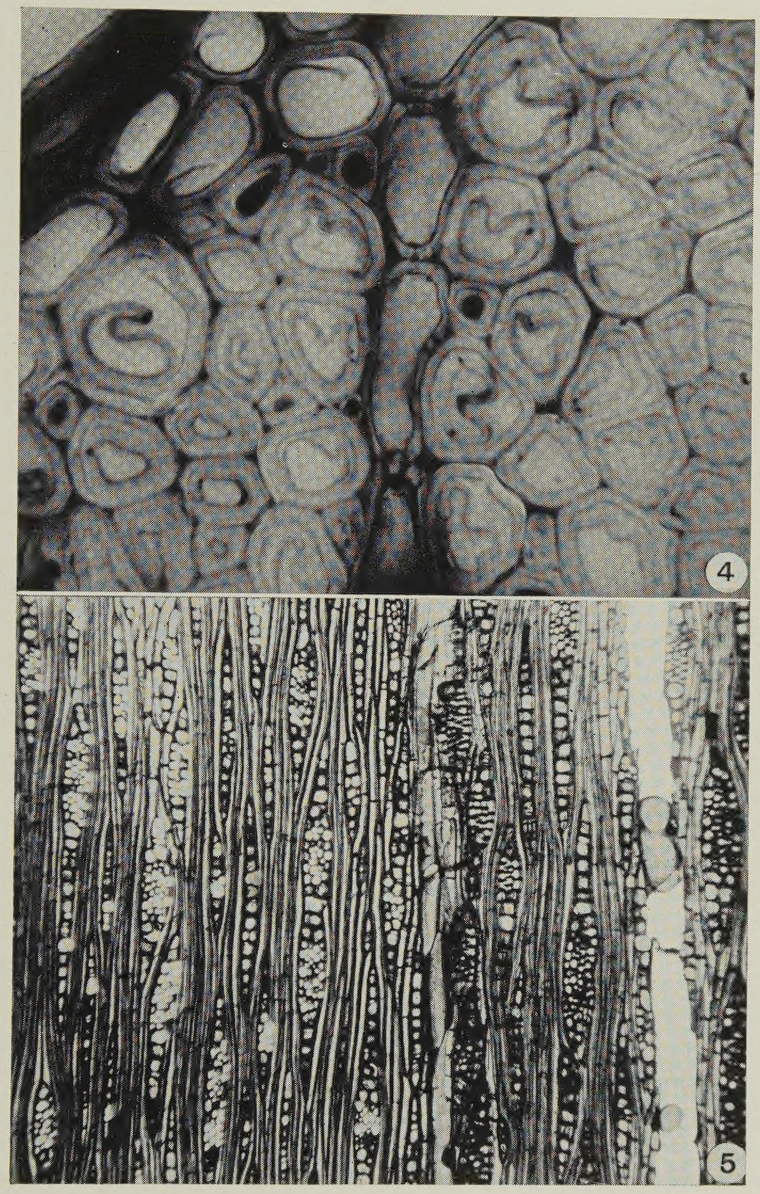

FIG. 4. *Celtis sinensis* Pers. (H9050). Transverse section (×1000), showing gelatinous libriform fibers and uniseriate ray with simple pits near center.

FIG. 5. *Celtis philippinensis* Blanco (H5370). Tangential section (×70), showing heterogeneous rays, both uniseriate and multiseriate; libriform wood fibers; and vessels with tyloses to the right, also vessels near the center.

Celtis tetrandra

Celtis tetrandra Roxb., also known as *Villebrunea integrifolia* Gaudich., is native to Burma, to India, and to central and eastern Himalayas (6). Only one specimen of this wood (H49047) was examined.

Growth rings are present. They vary from 3901 to 4980μ in width.

Libriform fibers are thick (lumen less than thickness of wall) to very thick-walled (lumen almost closed), gelatinous, and with small, rounded, simple pits. Lengths of fibers vary from 963 to 1926μ (av. 1428μ; mostly 1195 to 1577μ).

Vessels, averaging 44 per sq. mm. (mostly 30 to 50), are mostly (75%) in clusters of 3 to 18, or in multiples (25%) of 2 to 3. Very few solitary pores are found. Pores in cross-section are rounded to angular, Diameters in early wood vary from 183 to 332μ (av. 262μ; mostly 232 to 299μ); those in late wood from 50 to 166μ (av. 100μ; mostly 83 to 116μ). Walls of vessel elements are thin (less than 5μ). Lengths of the vessel elements measure from 199 to 398μ (av. 317μ; mostly 299–332μ). Perforations are simple, and the end walls oblique (35°) to transverse (few). Intervascular pits are numerous, medium-sized (7 to 10μ in diameter), rounded, and alternate. Vessel-parenchyma pits are alternate. A few thin-walled tyloses are found in some vessels and spiral thickenings in a few vessels. The wood is ring-porous.

Rays, averaging 6 per mm., are both uniseriate and multiseriate. The width of the multiseriate rays varies from 2 to 17 cells (mostly 10 to 14). The height of uniseriate rays numbers 2 to 27 cells (mostly 5 to 14); that of the multiseriates, from 12 to 136 cells (mostly 60–95). Pits are small, numerous, and simple. Crystals are present in ray cells. Type of ray is heterogeneous I, almost heterogeneous II A (Fig. 2).

Parenchyma is abundant and distribution is terminal (2 to 6 seriate), aliform, and confluent.

Comparison with other species.—Two of the woods, *C. sinensis* and *C. tetrandra,* described so far are ring-porous with growth rings; one, *C. philippinensis*, is diffuse-porous with only traces of growth rings. In *C. sinensis*, pores are mostly solitary or in clusters; in *C. philippinensis*, mostly solitary with a few multiples and in *C. tetrandra*, mostly clusters with a few multiples. Average length of fiber as well as length of vessel element is greater in *C. tetrandra* than in either of the other two woods. *C. sinensis* and *C. tetrandra* have a few vessel elements with transverse end walls and *C. philippinensis* has none that are entirely transverse. Multiseriate rays in *C. tetrandra* are wider and taller than any found in *C. philippinensis*, but very similar to those found in one specimen of *C. sinensis* (H9050), even to sheath cells. Terminal parenchyma is present in *C. sinensis* and *C. tetrandra;* confluent in all three species; aliform in *C. philippinensis* and *C. tetrandra;* and vasicentric in *C. sinensis* only.

Celtis crenata

Celtis crenata Planch., also known as *C. tala* Gill., is native to the Philippines. One specimen (H9040) was examined.

Only traces of growth rings are present in this diffuse-porous wood (Fig. 3).

Libriform fibers are thick (lumen less than thickness of wall) to very thick-walled (lumen almost closed), gelatinous, and with small, rounded, simple pits. Lengths vary from 598 to 1975μ (av. 1258μ; mostly 1062 to 1394μ).

Vessels, averaging 21 per sq. mm. (mostly 18 to 23), are solitary in distribution (80%) or in multiples of 2 to 10 (20%). Pores in cross-section are rounded to angular. Diameters vary from 66 to 199μ (av. 155μ; mostly 149 to 183μ). Walls of vessel elements are thin (less than 5μ). Lengths of vessel elements vary from 249 to 581μ (av. 400μ; mostly 349 to 498μ). Perforations are simple, end walls oblique (35°) to almost transverse (80°); intervascular pits numerous, rounded, medium-sized (7 to 10μ in diameter), and alternate. Vessel-parenchyma pits are also alternate. A very few thin-walled tyloses are present. Only a few vessel elements exhibit spiral thickenings.

Rays, averaging 7 per mm., are both uniseriate and multiseriate. The width of the multiseriate rays ranges from 2–6 cells (mostly 3 to 4). The height of uniseriate rays numbers from 2 to 24 cells (mostly 5 to 11); that of multiseriates from 9 to 47 cells (mostly 15 to 30). Pits to vessels are small, numerous, and simple. Numerous crystals are in the ray cells. Ray type is heterogeneous I.

Parenchyma is abundant; distribution is terminal (1 to 3-seriate), metatracheal, vasicentric, aliform and confluent.

Comparison with other species.—*C. crenata*, like *C. philippinensis*, is diffuse-porous with only traces of growth rings (Fig. 3), and has about the same number of pores per sq. mm. The distribution of pores is likewise similar to *C. philippinensis*, mostly (80%) solitary or in multiples (20%). Average length of vessel element is greater in *C. crenata* than that in any of the other woods examined. Length of fiber is second only to *C. tetrandra*. The two ring-porous woods have some vessel elements with transverse end walls; the two diffuse-porous woods have a few that are almost transverse. Multiseriate rays in *C. crenata* and *C. philippinensis* are shorter and not as wide as those in *C. sinensis* and *C. tetrandra*. Both ring-porous woods have terminal and confluent parenchyma. In addition, *C. sinensis* has vasicentric, while *C. tetrandra* has aliform. Both diffuse-porous woods have aliform and confluent parenchyma. In addition, *C. crenata* has terminal, metatracheal, and vasicentric.

DISCUSSION

The woods of the four species of Celtis studied can be distinguished anatomically as follows:

Celtis sinensis and *Celtis tetrandra* are ring-porous. The former has shorter fibers, shorter vessel elements, somewhat smaller and more numerous pores than the latter. Pore distribution in *C. sinensis*, is mostly solitary or in clusters, with a few multiples. Pores in *C. tetrandra* are mostly in clusters or multiples with few solitary pores. Rays are wider and taller in *C. tetrandra*.

Celtis philippinensis and *C. crenata* are both diffuse-porous with pore distribution mostly solitary or in multiples. There are more pores in multiples, however, in *C. crenata*. Pores in the former are round and those in the latter round to angular. *C. crenata* has spiral thickenings and longer average lengths for fibers and for vessel elements.

It should be emphasized that only one specimen of two of the species was available for study. It is entirely possible that if more samples were studied some of these differences might disappear.

Little variation was found in the four specimens of *C. sinensis* which were examined. Only one specimen (H9050) has sheath cells. Both uniseriate and multiseriate rays are taller in this specimen than those in the other three. Practically no differences were noted between the two specimens of *C. philippinensis*. However, one specimen (H5370) has thick-walled tyloses. Only one specimen was examined of each of the other two species.

SUMMARY

1. The anatomy of the secondary xylem of *C. sinensis* Pers., of *C. philippinensis* Blanco., of *C. tetrandra* Roxb., and of *C. crenata* Planch., is described.
2. These species can be distinguished on the basis of their wood anatomy.
3. Anatomical variation within a species is limited to a few minor differences.

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